

# WRIGHT STATE

Wright State University  
Dayton, Ohio 45435

US EPA CHICAGO REGIONAL LAB.  
533 S. CLARK ST.  
CHICAGO, ILLINOIS 60603

N  
N.D  
153 766  
Brehm Laboratory

513/873-2202

Data

Transmitted  
3/25/82

March 16, 1982

by CK  
to Dan

Wagner

Mr. Curtis Ross  
United States Environmental Protection Agency  
Region V  
230 S. Dearborn  
Chicago, Illinois 60604

Re: EPA Order #56606NAEX

Dear Mr. Ross:

As you know, the samples of leachate received in our laboratory on January 14, 1982 under the subject EPA Order No., have been analyzed for chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs), and these data were reported to you in a telephone conversation by Dr. T.O. Tiernan on February 25, 1982. The purpose of this interim report is to confirm in writing the data on CDDs/CDFs which was verbally transmitted as indicated above, and to provide you with the details of the analytical procedures employed, as well as copies of the original Gas Chromatographic-Mass Spectrometric data obtained in these analyses.

Table 1, which is attached, lists the samples which were received, along with a brief description of each of the samples. Table 2, which is also attached, indicates the concentrations of CDDs/CDFs determined to be present in each of the five water samples which were submitted by EPA. As can be seen from the data in Table 2, no detectable tetrachlorodibenzo-p-dioxins (TCDDs), tetrachlorodibenzofurans (TCDFs), pentachlorodibenzo-p-dioxins (PCDDs) or pentachlorodibenzofurans (PCDFs) were found in these aqueous samples. However, higher chlorinated dioxins and furans [hexachlorinated dibenzo-p-dioxins (HxCDDs), hexachlorodibenzofurans (HxCDFs), heptachlorodibenzo-p-dioxins (HpCDDs), heptachlorodibenzofurans (HpCDFs), octachlorodibenzo-p-dioxin (OCDD), and octachlorodibenzofuran (OCDF)] were detected in three of the five samples. The concentrations of the higher chlorinated CDDs/CDFs ranged from 4.5 picograms/ml (4.5 parts-per-trillion, 4.5 ppt) to 2,693 ppt, among the three samples which were found to contain CDDs/CDFs. Whether or not the CDDs/CDFs are present as solutes or are associated with suspended particulate matter in these samples cannot be determined on the basis of the present results. Copies of the original data obtained in these determinations are appended to this interim report (see Attachment 1). Figures showing mass chromatographic data pertinent to each of the 5 samples, as well as representative GC-MS results obtained for calibration standards, are included in each of the six sections in Attachment 1. Labelling of the

Mr. Curtis Ross  
March 16, 1982  
Page 2

figures for this report has been abbreviated to permit rapid submission of this data. At the top of each figure are listed the nominal masses which correspond to the respective analog signals displayed in that figure. By referring to Table 3, one can determine the ion masses monitored as indicators of each class of CDDs/CDFs.

Invariably, analyses of complex environmental samples for CDDs and CDFs is a research project until the optimum technique is developed. Although an established Brehm Laboratory protocol has been developed for preparing and analyzing aqueous samples similar to those submitted by EPA under this Order No., initial attempts to implement these "established" methods yielded results which were not acceptable. This indicated the need to modify the established procedures in order to adequately analyze these specific samples. The analytical methodology employed for these analyses is outlined in detail in Attachment 2. However, a few general comments are in order regarding the procedures employed. Initially, each of the approximately 3000 mL samples were agitated to suspend fine particulate matter contained therein in an effort to ensure sample homogeneity. A 100 mL aliquot of the sample was immediately removed at this point, and transferred to a 250 mL precleaned flint glass bottle, equipped with a Teflon-lined cap. Microliter quantities of solutions containing the internal standards utilized in these analyses,  $^{37}\text{Cl}_4$ -2,3,7,8-TCDD,  $^{37}\text{Cl}_4$ -1,2,3,4,6,7,8-HpCDD, and  $^{37}\text{Cl}_8$ -OCDD, were then added to each of the samples. Subsequently, the samples were prepared and analyzed as described in Attachment 2. It must be emphasized that not all of the possible CDDs/CDFs isomers are on hand in this laboratory or in any other laboratory at present. Hence, the analytical procedures described, have not been rigorously tested using all of the 136 CDDs/CDFs which comprise the tetra- thru octachlorinated dibenzo-p-dioxins and furans. However, all 22 TCDD isomers, as well as at least one of the other CDD/CDF isomers from each chlorinated class of CDDs/CDFs, are available in the Brehm Laboratory for calibration purposes. The calibration of the GC-MS-DS system also deserves special comment. As indicated above the CDDs/CDFs standards on hand were employed to obtain gas chromatographic retention time data and mass spectral data which typify a particular class of CDDs/CDFs. These data were used as the basis for establishing the GC-MS conditions appropriate for detecting and quantitating a particular class of CDDs/CDFs. As indicated above, ideally all isomers comprising a particular class of CDDs/CDFs should be available so that the retention times and mass spectral cracking patterns for each isomer could be determined. However all isomers are not available (except in certain cases such as the TCDDs) and therefore the isomers which are available of necessity are regarded as representative of the entire class. This approach may, of course, be subject to certain errors, for example, the gas chromatographic and mass spectrometric response of each isomer of a given class of CDDs/CDFs undoubtedly is slightly different and therefore one can only estimate what the retention times for each member of a particular class may be. This estimate is based in part on previous experience, and in part on other available data (for example, the relative GC retention times for other isomers in a given class). On these bases a retention time window is selected which is reasonably expected to encompass the retention times of all isomers in that class. Regarding the mass spectral behavior of isomers in a given class, it should be noted that the mass spectral ion-masses which are monitored for the quantitation of the various CDDs/CDFs all correspond to the molecular ion for that isomer (or a peak in the molecular ion isotopic cluster).

The error which may be inherent in this practice is that all isomers likely exhibit slightly different molecular ion fragmentations. However, these differences are not expected to be great (no greater than a factor of 2 difference between isomers). Finally, the actual quantitation of each CDD/CDF class is accomplished by using an internal standard technique, that is, the signal at an ion-mass typical of a particular CDD/CDF class is acquired and the peak area is obtained. The ratio of this area to the area obtained for the corresponding internal standard is calculated and the quantity of the class of CDDs/CDFs present in the sample is then calculated, taking into account the volume of extract injected, the total volume of the extract and the quantity of sample prepared for analysis. In the case of the HpCDDs, where 2 isomers are possible, the peak areas for each of these are summed during the analysis and the summed area relative to the peak area for the internal standard is obtained and the calculation of the concentration is performed as described above. In the case of the TCDFs where 38 isomers are possible, up to 38 peaks could be obtained (if it were possible to chromatographically separate all 38 isomers) and the areas of these peaks would be summed and their ratio to the internal standard would be obtained. This approach represents the current state-of-the-art of ultratrace analysis of CDDs/CDFs. No laboratory in the world possesses all of the possible CDDs/CDFs isomers and hence each laboratory can only accomplish calibrations using the standards available to that laboratory. It must be noted too, that several laboratories have unauthenticated standards or mixtures of these, (isomers which have been synthesized and identified solely on the basis of the predicted or expected products from the synthetic reaction) which are employed as calibration standards. This practice must be viewed with serious reservation until identity of the standards is confirmed either by X-ray crystallographic analyses or other interlaboratory comparative analyses.

Table 3 lists the three  $^{37}\text{Cl}$ -labelled internal standards which were employed throughout the course of these analyses. These standards, being chemically identical to the corresponding native CDDs, afford an excellent means of assessing the efficacy of the analytical methodology employed. A comparison of the quantity of each of these standards which is added to a given sample in known amount prior to processing with the quantity of this standard actually recovered is indicative of the overall reliability of the analysis. Analyses in which the recoveries of the internal standard employed were less than 50% were generally repeated (with slight modifications of methodology) until satisfactory recovery was achieved. The  $^{37}\text{Cl}$ -labelled compounds were employed here as true internal standards, that is, the concentrations of the native compounds were determined from the ratio of the ion signal for the material to that for the added internal standard. The data listed in Table 2 are corrected for recoveries, therefore. Work is still in progress in our laboratory to characterize other organic components which may be present in these samples, as specified in the EPA work statement. These analyses should be completed and the data reported to EPA within the next two weeks.

Mr. Curtis Ross  
March 16, 1982  
Page 4

We appreciate this opportunity to work with you and EPA. If you have questions regarding these data please don't hesitate to call us.

Sincerely,



Thomas O. Tiernan, Ph.D.  
Professor of Chemistry and  
Director of Brehm Laboratory



Michael L. Taylor, Ph.D.  
Associate Professor of  
Pharmacology/Toxicology and  
Associate Director of  
Brehm Laboratory

TOT/gdg

Enclosures

TABLE 1

BREHM LABORATORY, WRIGHT STATE UNIVERSITY, DAYTON, OHIO 45435LISTING OF SAMPLES RECEIVED FROM USEPA (CHICAGO, REGION V)<sup>1</sup>.

<u>EPA I.D. No.</u>	<u>WSU Sample No.</u>	<u>Description</u>
E1205 82WT06S01	CWS-1	1 gallon of water/sediment
E1206 82WT06S03	CWS-2	3/4 gallon of water/sediment
E1208 82WT06S05	CWS-3	1 gallon of water/sediment
E1207 82WT06S07 82WT06R01	CWS-4 CWS-5 <i>3 Blanks</i>	3/4 gallon of water/sediment 3/4 gallon of water/sediment

<sup>1</sup>. Samples were received on January 14, 1982. Samples were packed in styrofoam beads, and ice water was present in shipping containers. Samples CWS-2 and CWS-5 were shipped together in one container and samples CWS-1,-3 and -4 were shipped together in a second container. Caps on bottles were taped.

TABLE 2

BREHM LABORATORY, WRIGHT STATE UNIVERSITY, DAYTON, OHIO 45435

CONCENTRATIONS OF TETRA- THRU OCTACHLORINATED DIBENZO-p-DIOXINS AND DIBENZOFURANS  
IN LEACHATE RECEIVED FROM USEPA

WSU Sample No.	CDDs/CDFs in Parts-Per-Trillion (limits of detection in parentheses)									
	TCDDs	TCDFs	PCDDs	PCDFs	HxCDDs	HxCDFs	HxCDDs	HxCDFs	OCDD	OCDF
CWS-1	0(1)	0(1)	0(2)	0(2)	4.5	6.3	86	74	323	30
CWS-2	0(1)	0(1)	0(1)	0(1)	6.3	10	181	182	675	103
CWS-3	0(1)	0(1)	0(2)	0(2)	5.8	6.3	152	112	2,693	53
CWS-4	0(1)	0(1)	0(2)	0(2)	0(3)	0(3)	0(4)	0(4)	0(6)	0(6)
CWS-5 (Field Blank)	0(1)	0(1)	0(2)	0(2)	0(3)	0(3)	0(3)	0(3)	0(6)	0(6)

Note: See Table 1 for EPA ID match up with  
 WSU sample # system C.Raw 3/25/82

TABLE 3

BREHM LABORATORY, WRIGHT STATE UNIVERSITY, DAYTON, OHIO 45435LISTING OF NOMINAL AND EXACT MASSES MONITORED FOR EACH CLASS OF CDDs/CDFs

<u>CDDs/CDFs</u>	<u>Exact Mass</u>	<u>Nominal Mass</u>
TCDDs	319.897 321.894 327.885 <sup>a.</sup>	320 322 328
TCDFs	303.902 305.899	304 306
PCDDs	353.858 355.855	354 356
PCDFs	337.863 339.860	338 340
HxCDDs	389.816 391.813	390 392
HxCDFs	373.821 375.818	374 376
HpCDDs	423.777 425.774 <sup>b.</sup> 431.765	424 426 432
HpCDFs	407.782 409.779	408 410
OCDD	457.738 459.735 471.717 <sup>c.</sup>	458 460 472
OCDF	441.743 443.740	442 444

<sup>a.</sup>Corresponds to  $^{37}\text{Cl}_4\text{-}2,3,7,8\text{-TCDD}$  employed as an internal standard for TCDDs, TCDFs, PCDDs, PCDFs, HxCDDs and HxCDFs.

<sup>b.</sup>Corresponds to  $^{37}\text{Cl}_4\text{-}1,2,3,4,6,7,8\text{-HpCDD}$  employed as an internal standard for HpCDDs and HpCDFs.

<sup>c.</sup>Corresponds to  $^{37}\text{Cl}_8\text{-OCDD}$  employed as an internal standard for OCDD and OCDF.

ATTACHMENT 1

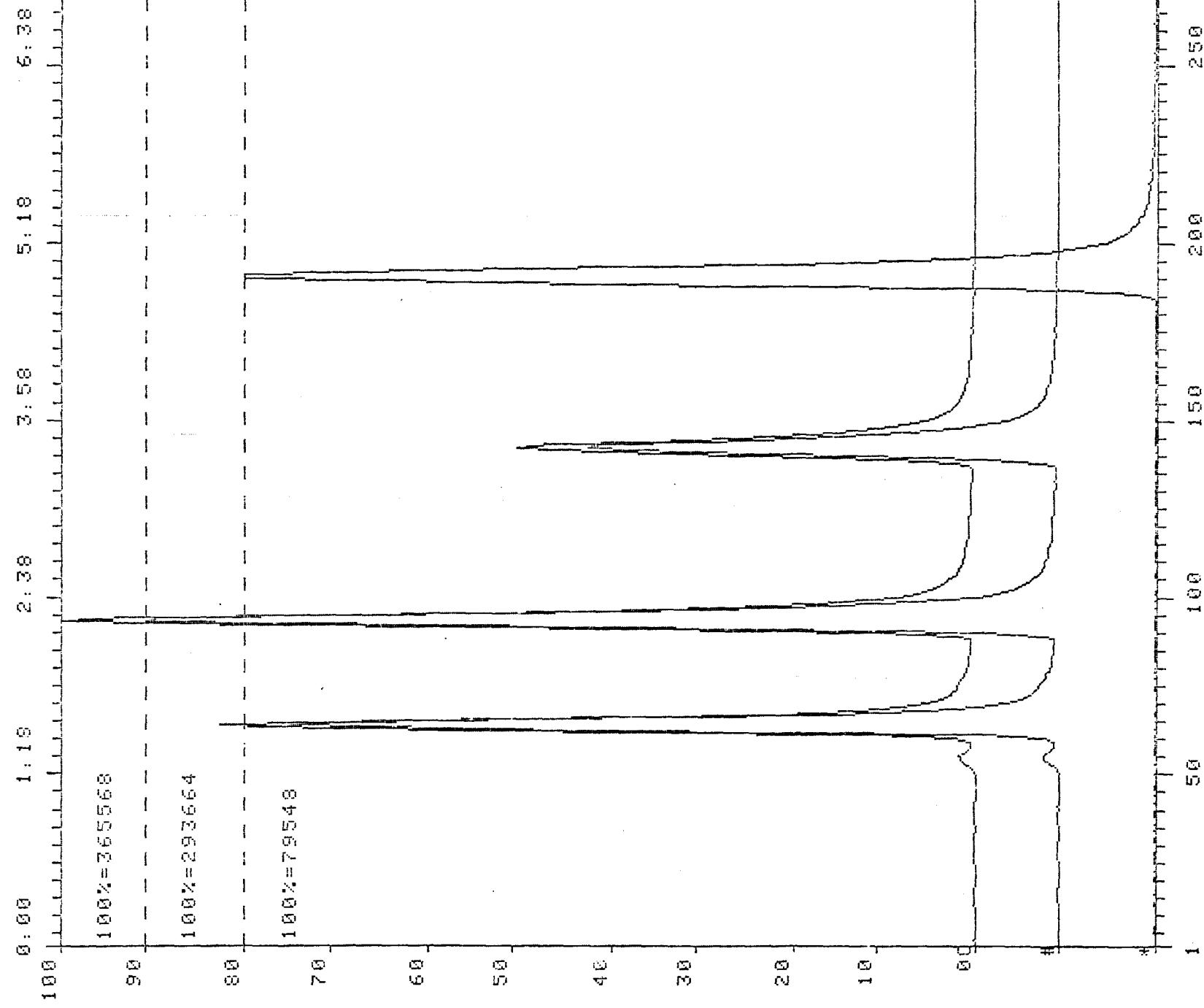
GC-MS SELECTED-ION MASS CHROMATOGRAMS

OBTAINED FOR USEPA LEACHATE SAMPLES

A. CDD and CDF Standards

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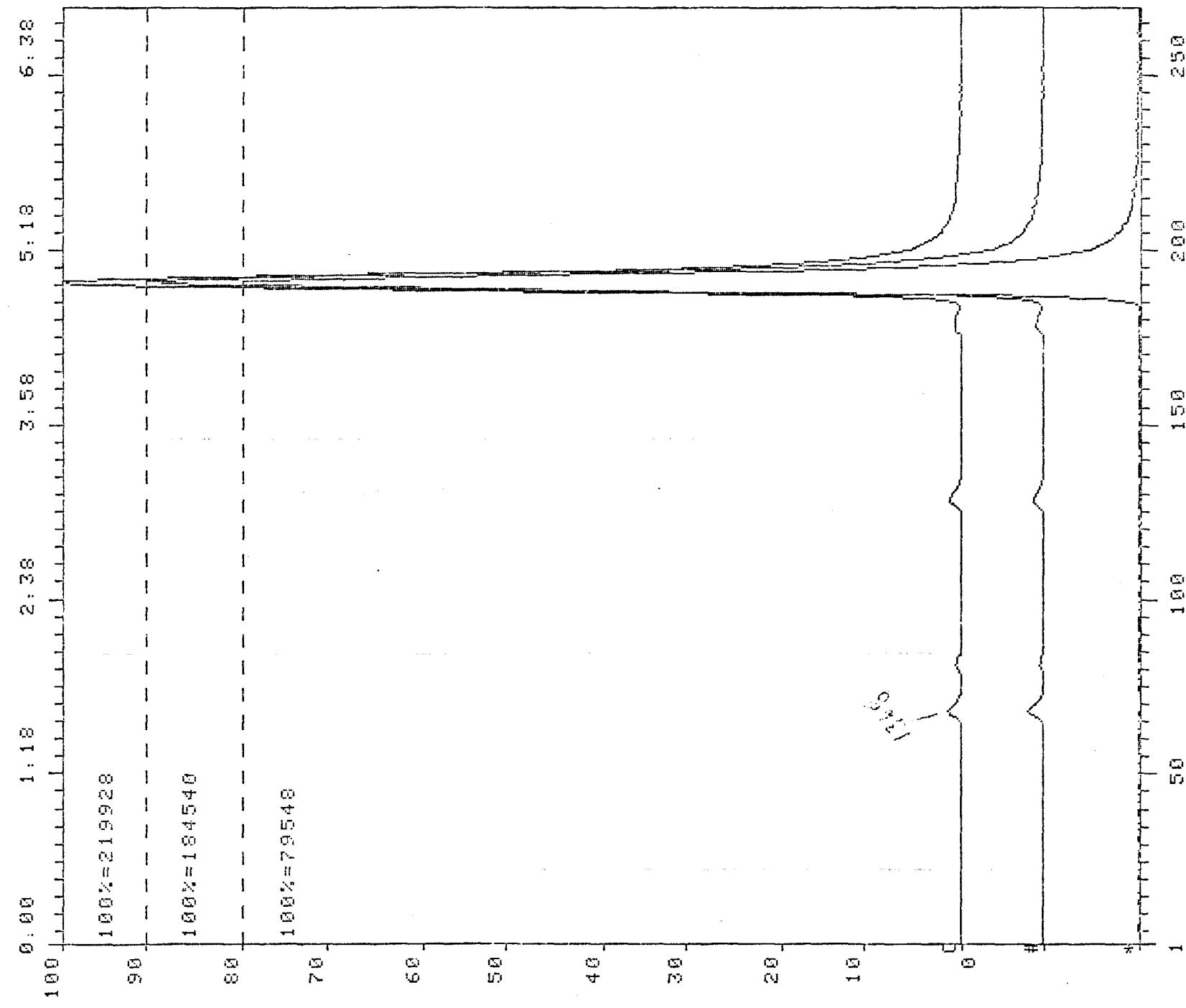
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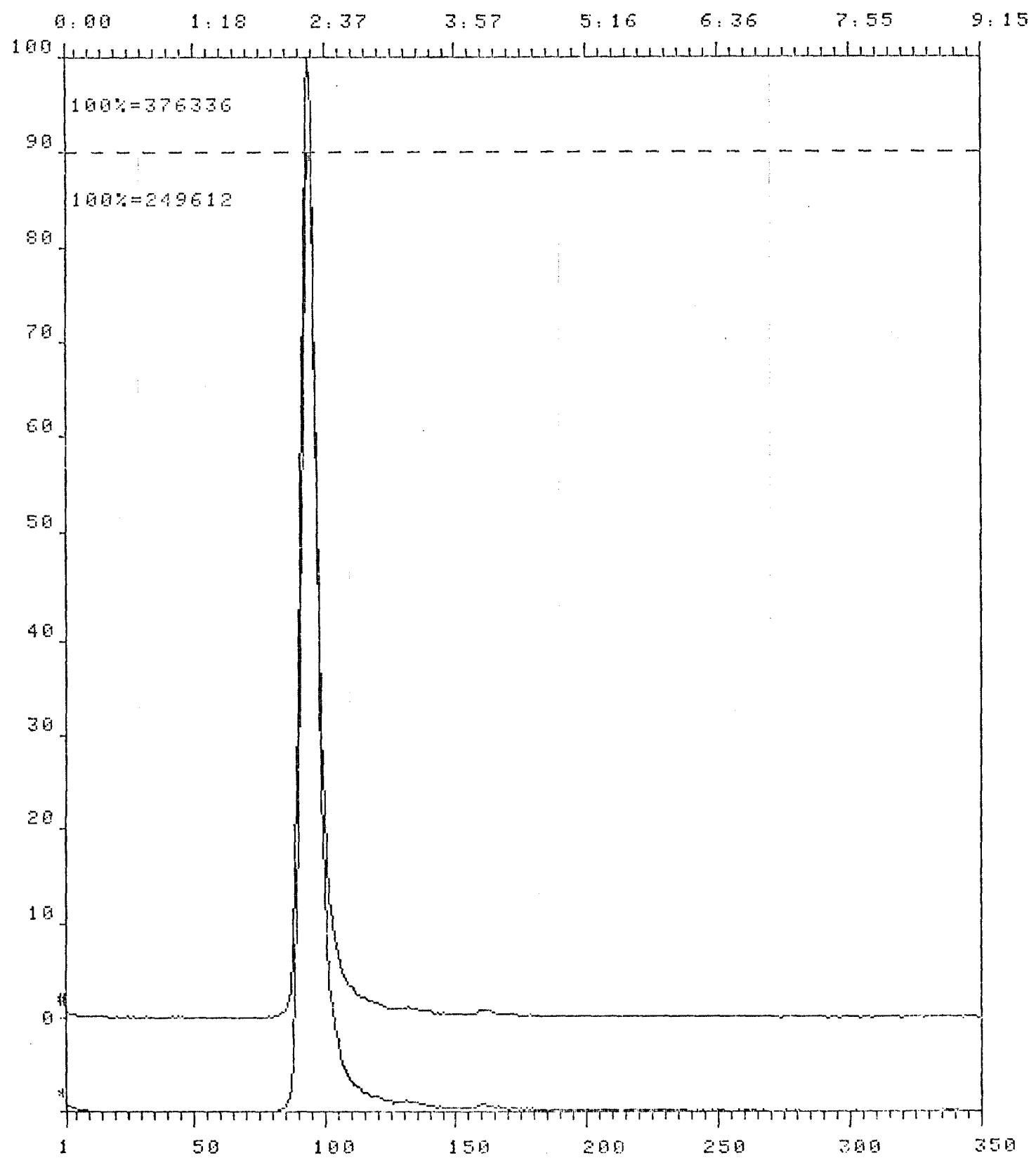
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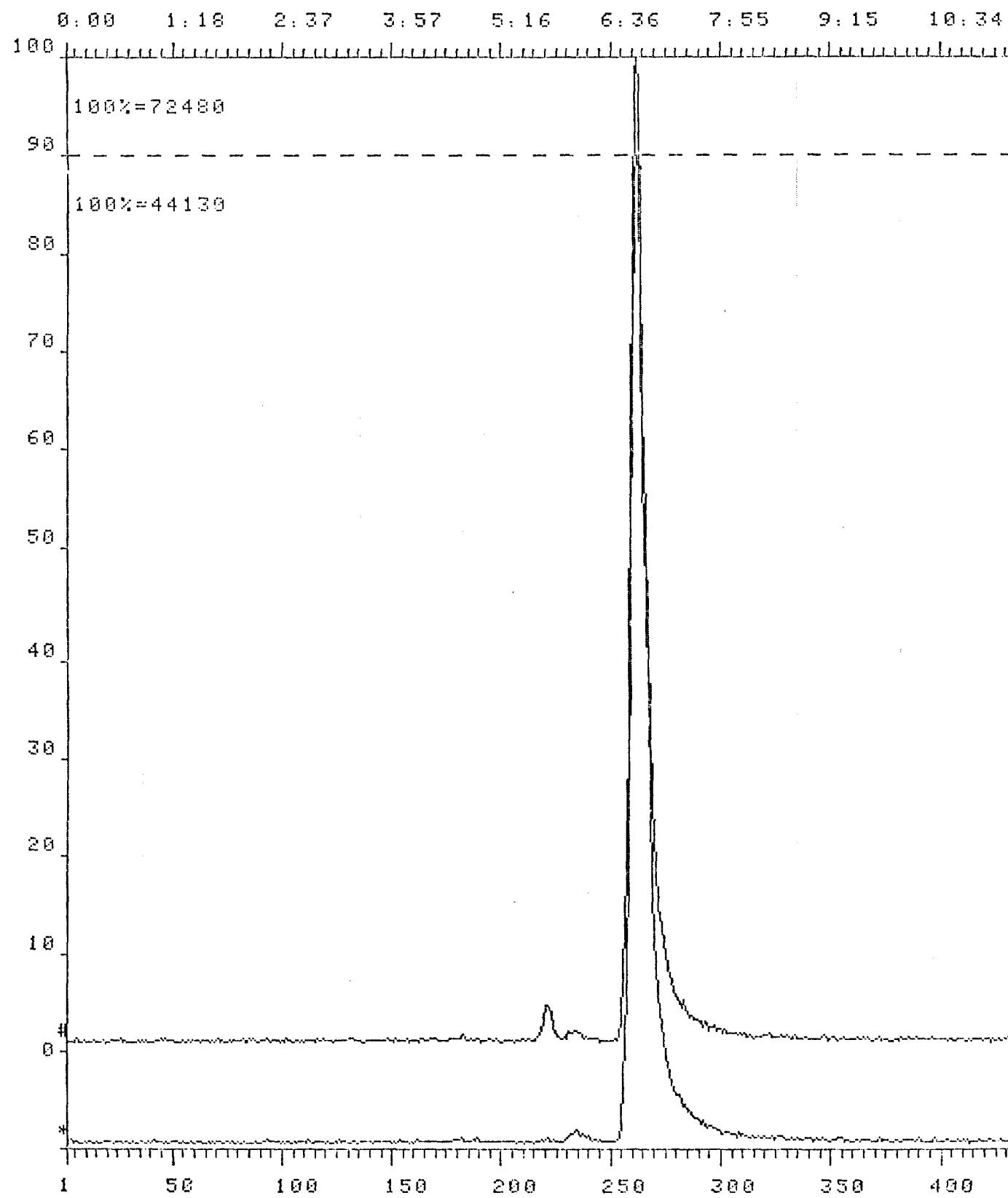
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\* 338 # 340



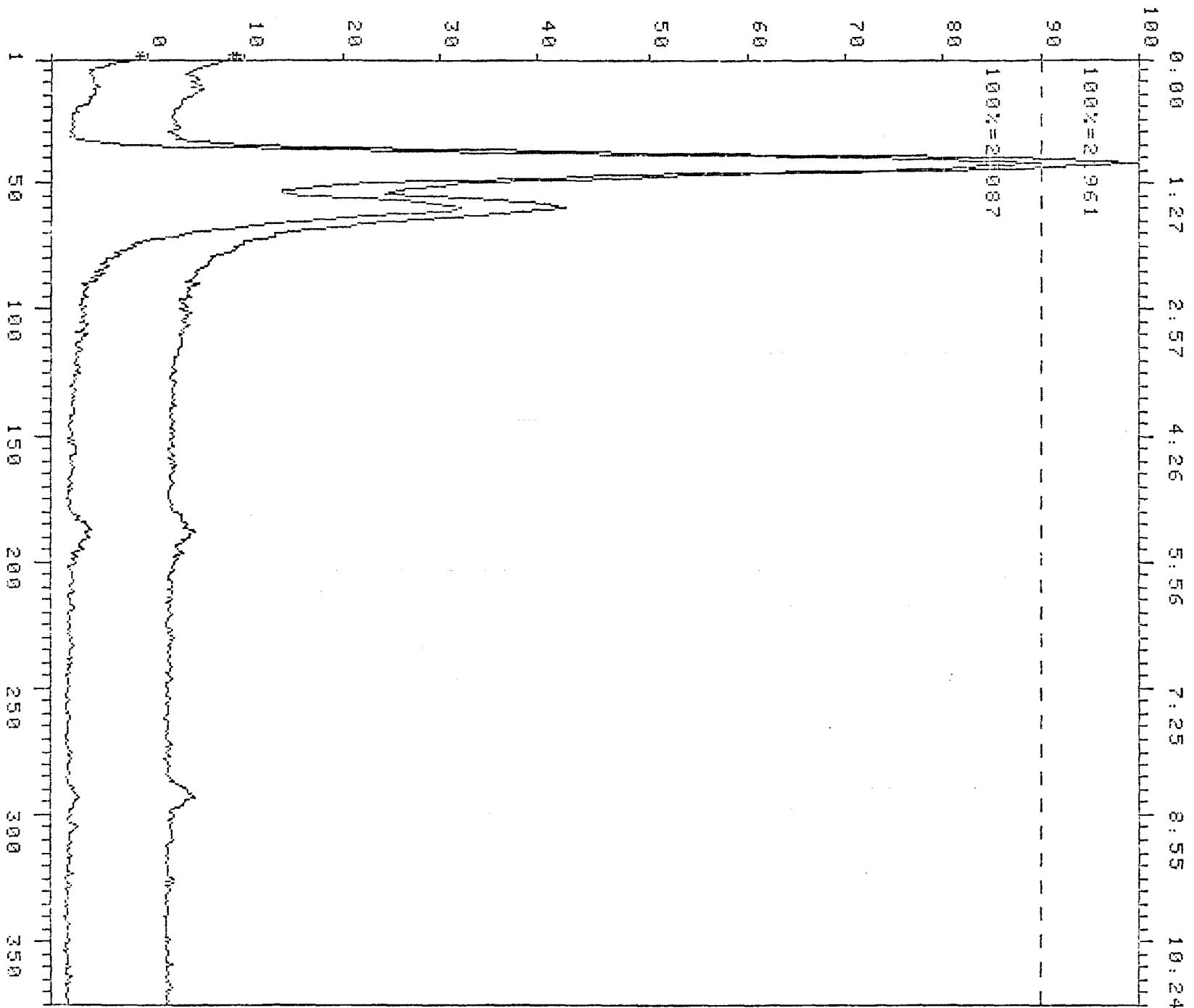
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\* 354 # 356



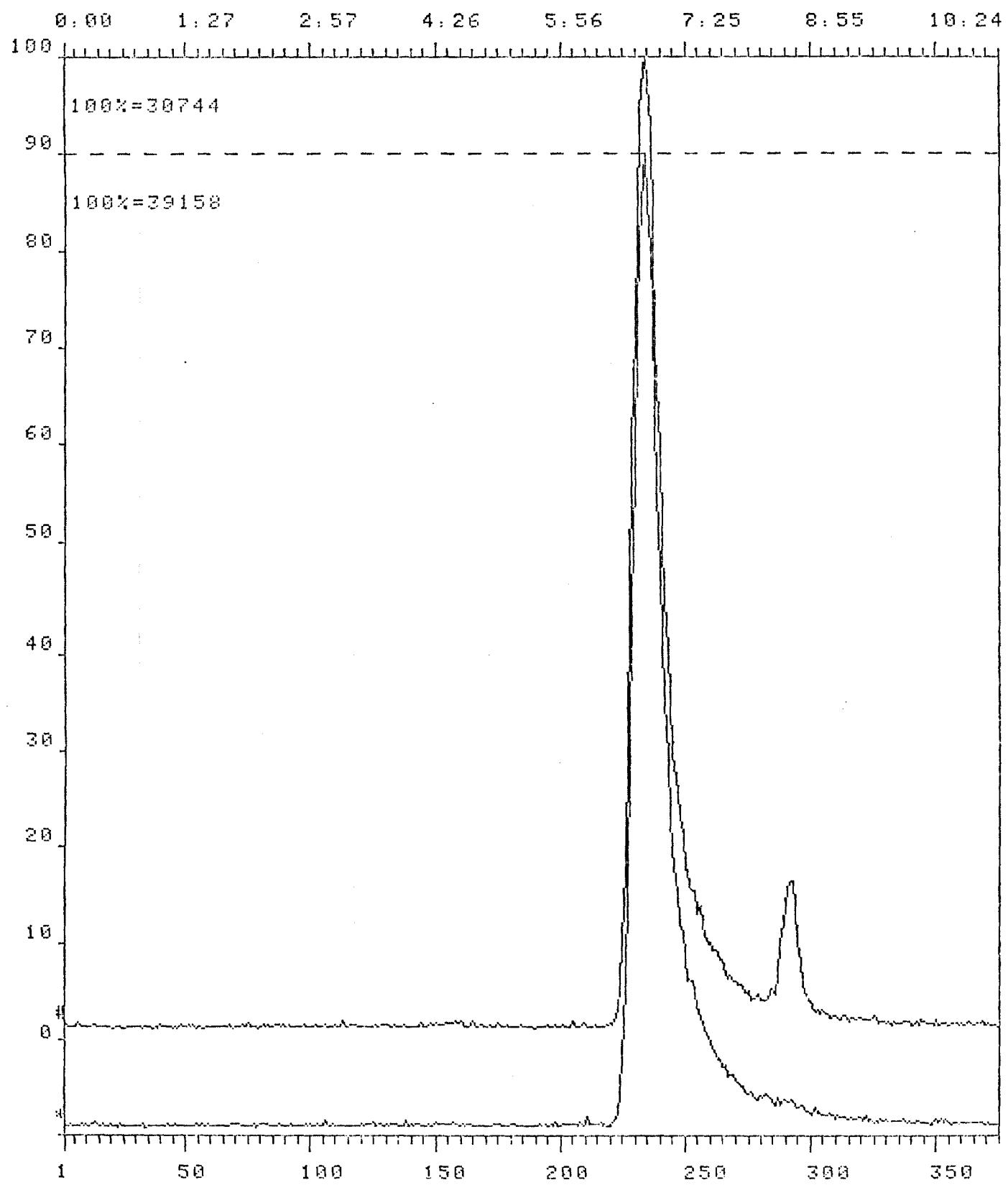
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\* 374 # 376



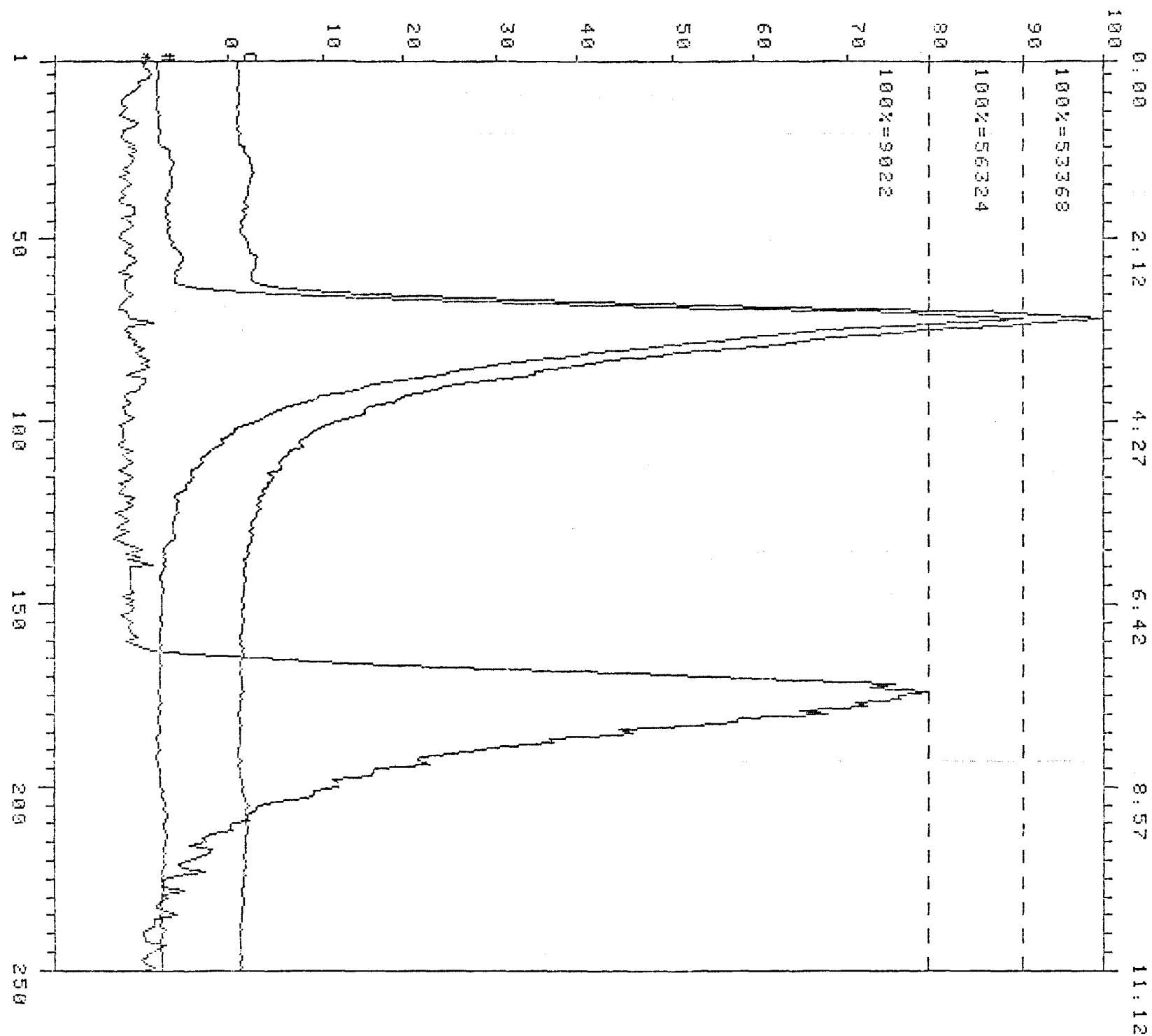
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\* 390 # 392



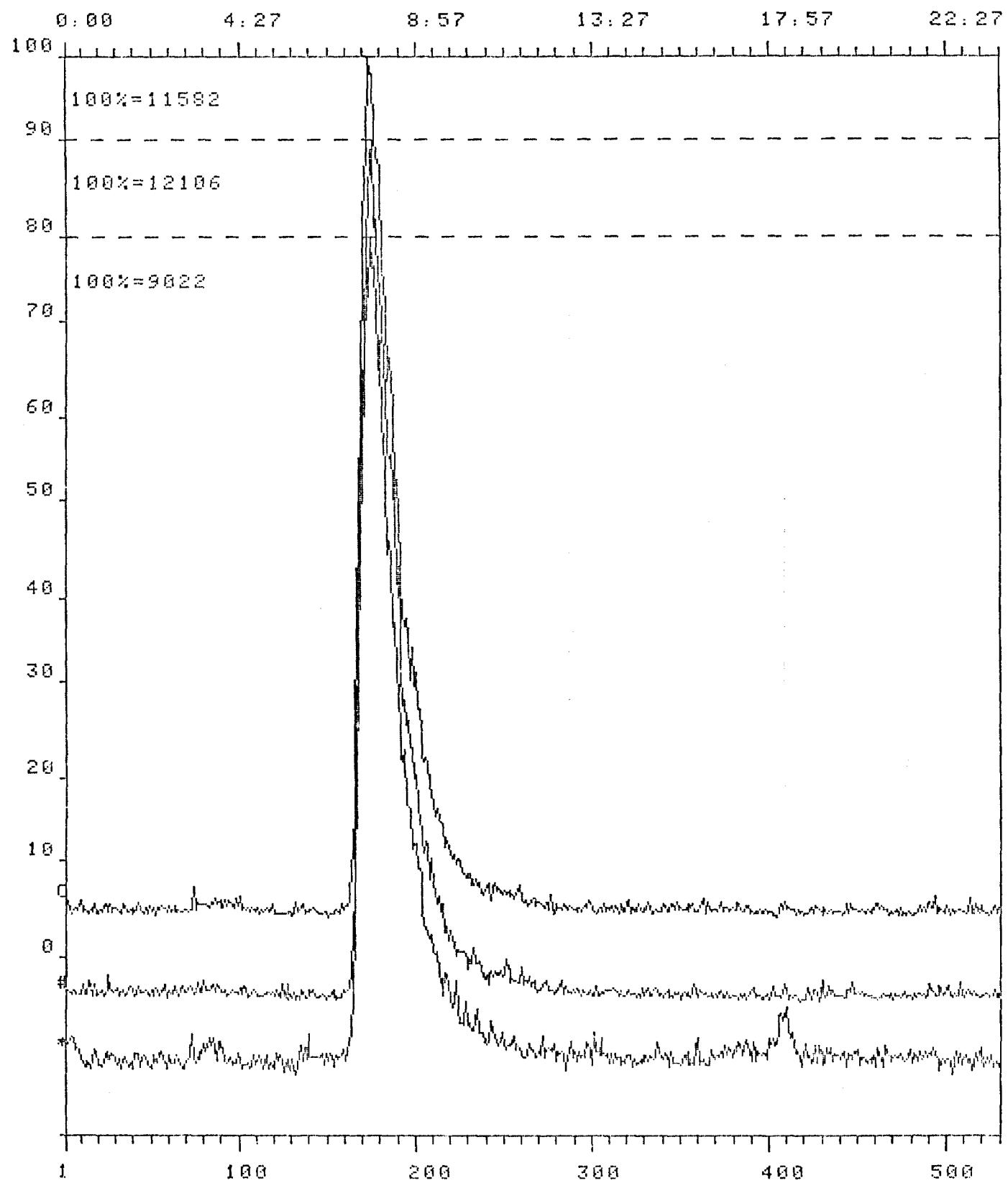
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\* 432 # 408 0 410



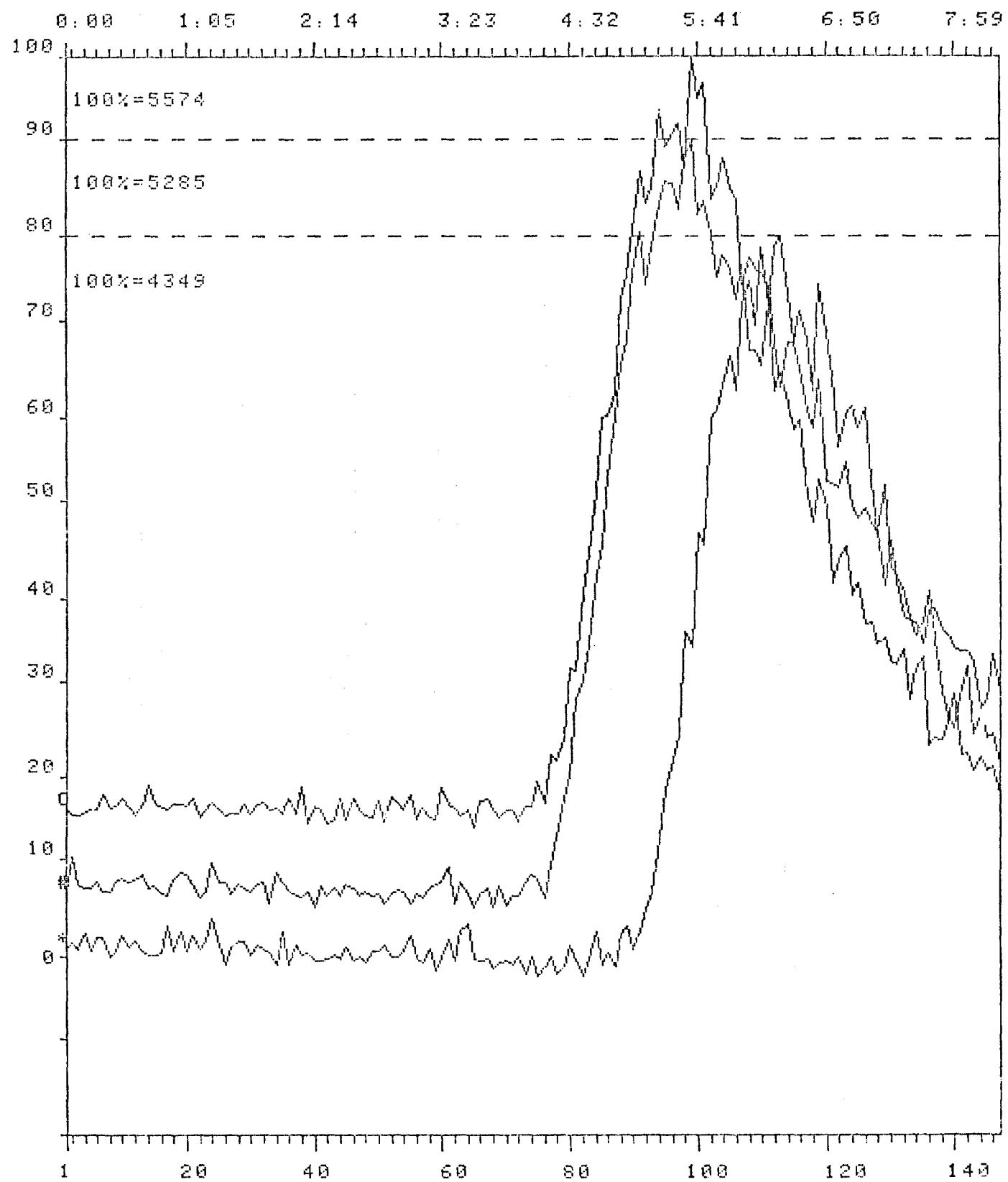
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\* 432 # 424 O 426



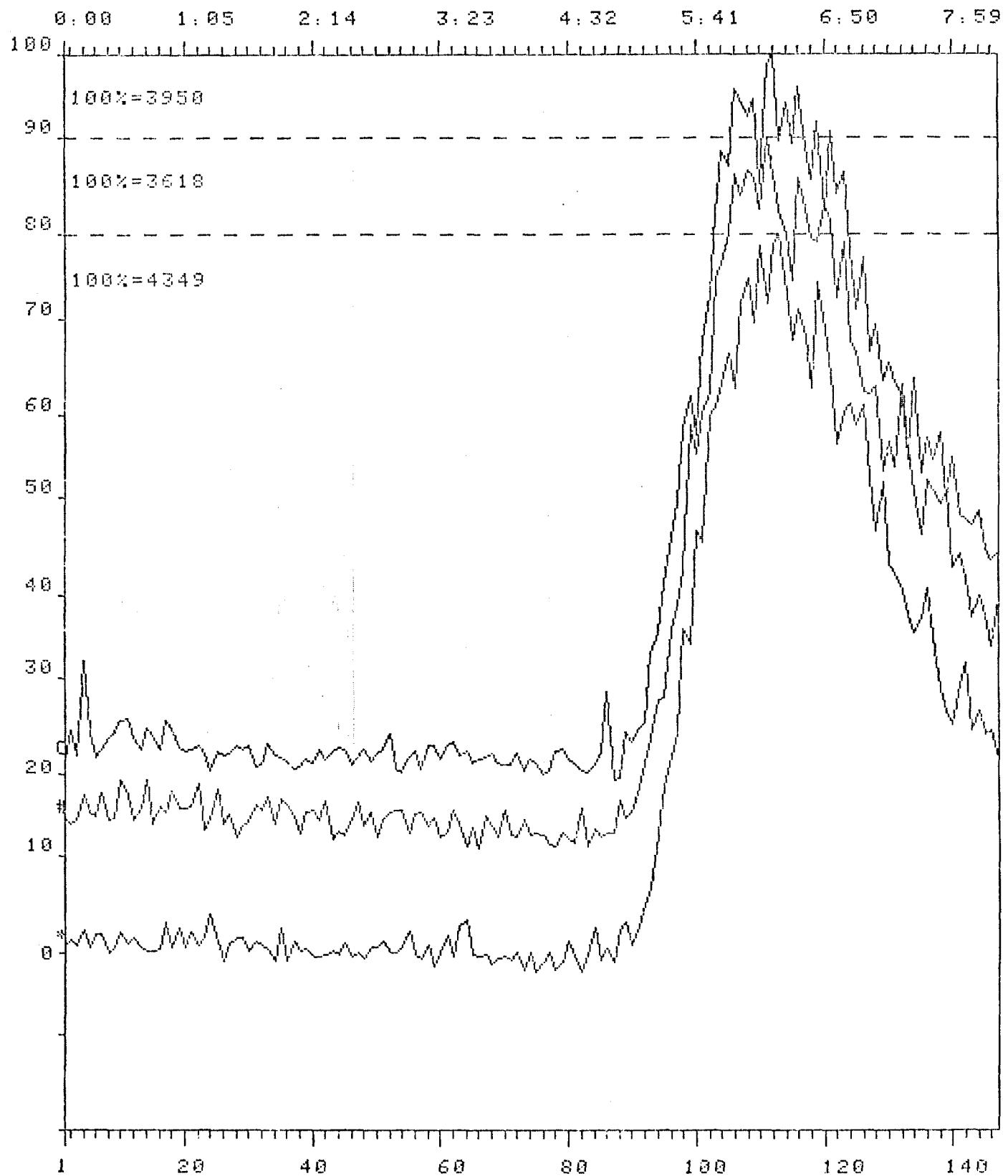
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\* 472 # 442 0 444

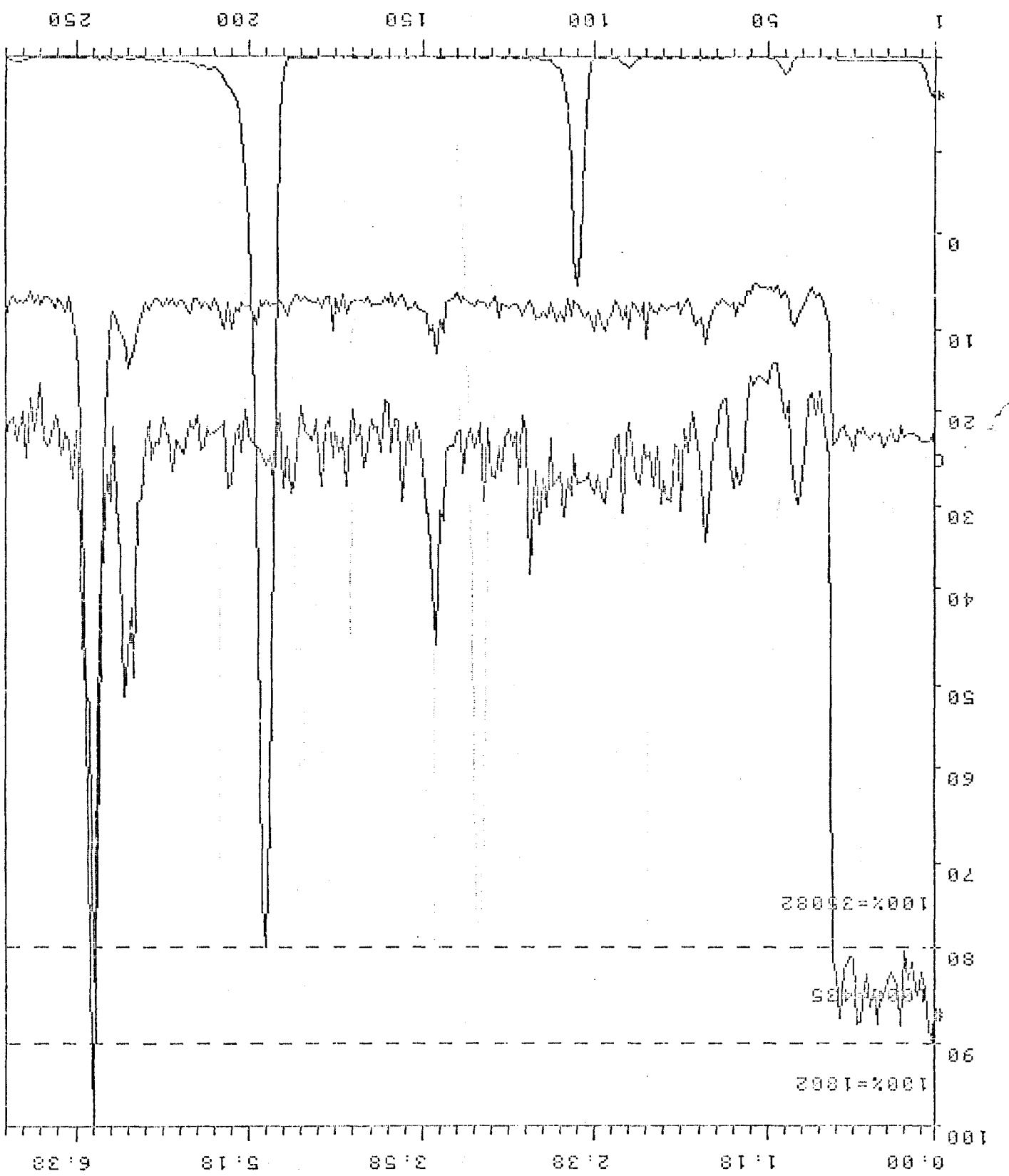


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\* 472 # 458 0 460



B. CWS-1

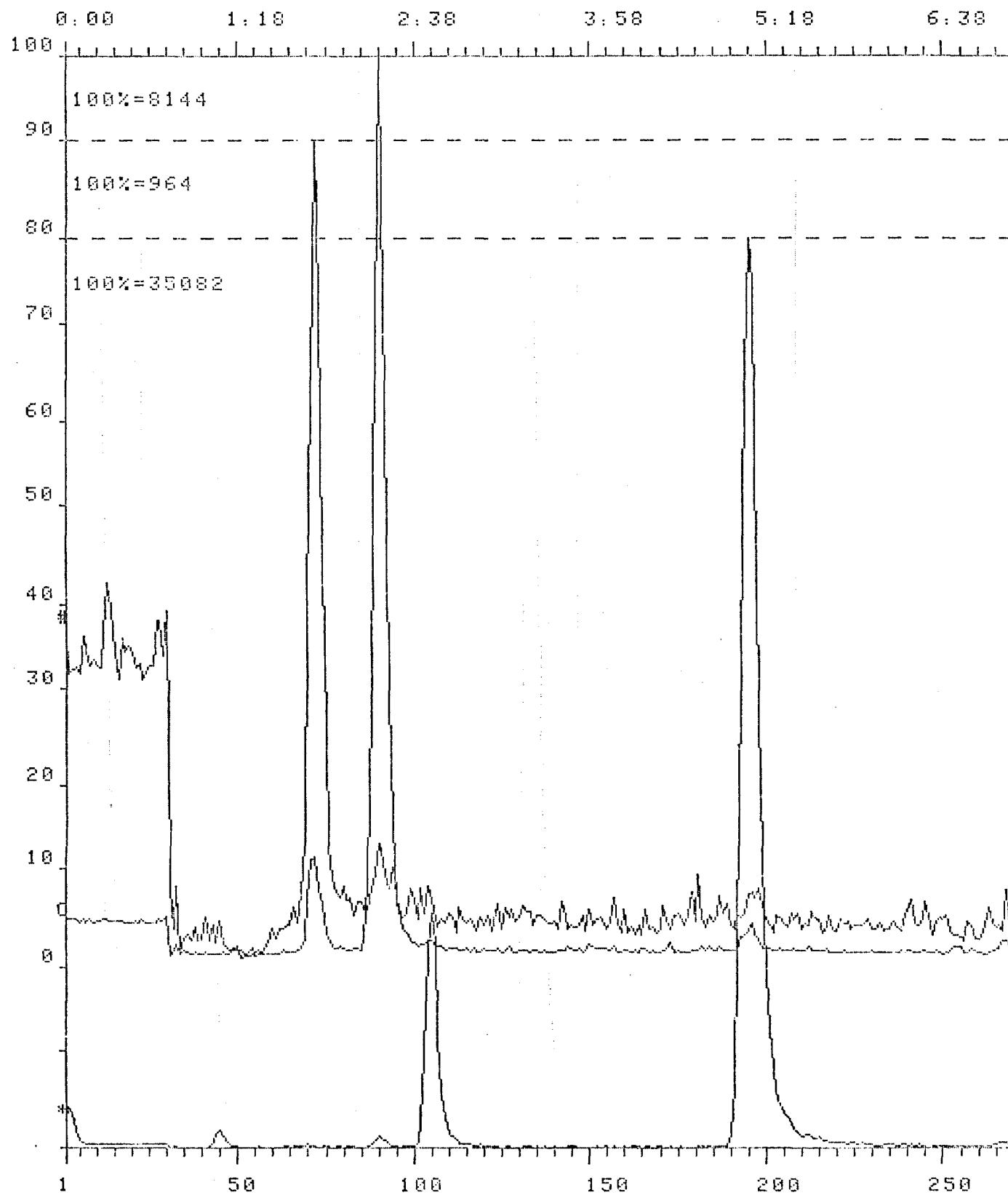


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\* 328 # 304 0 306

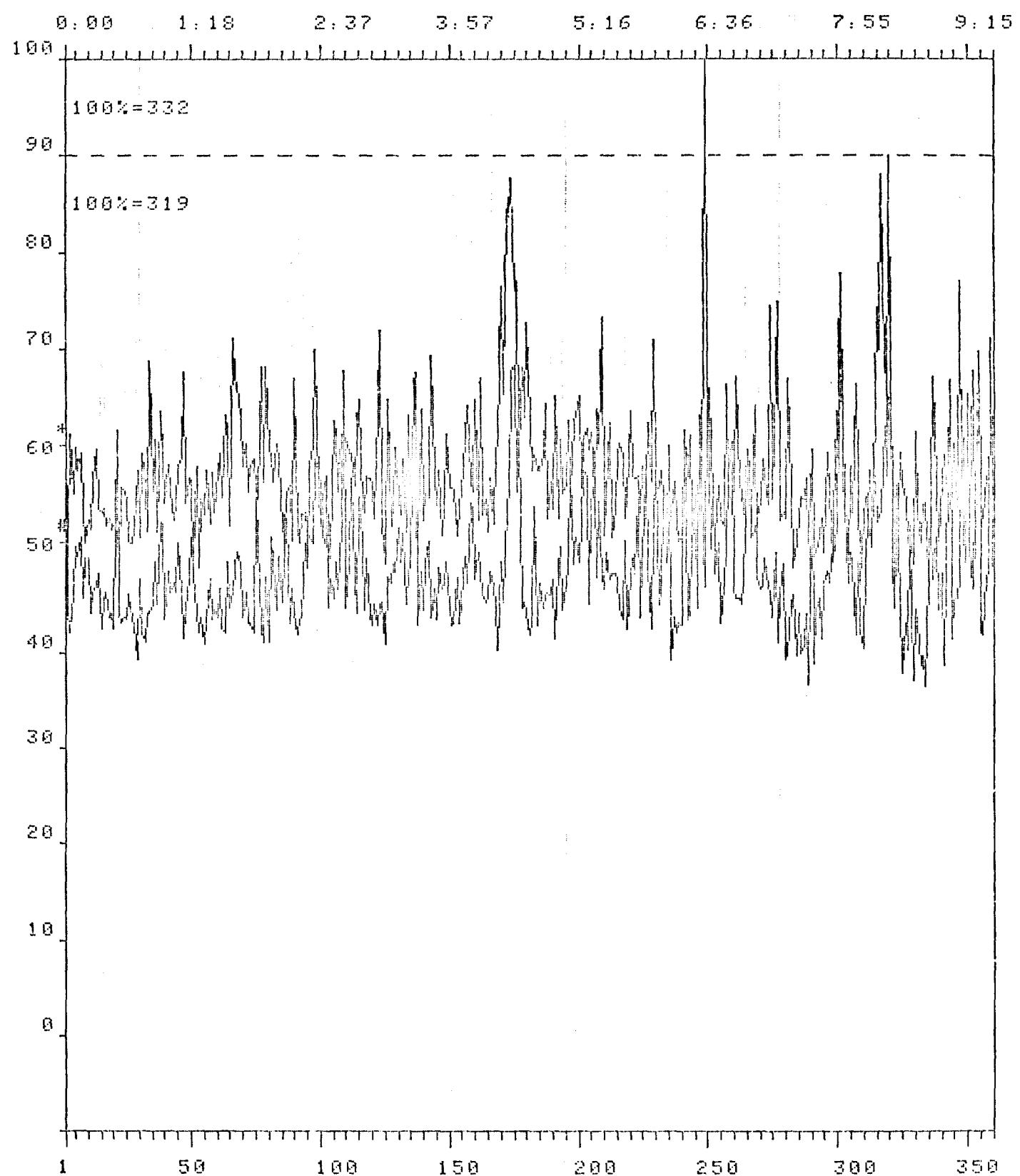
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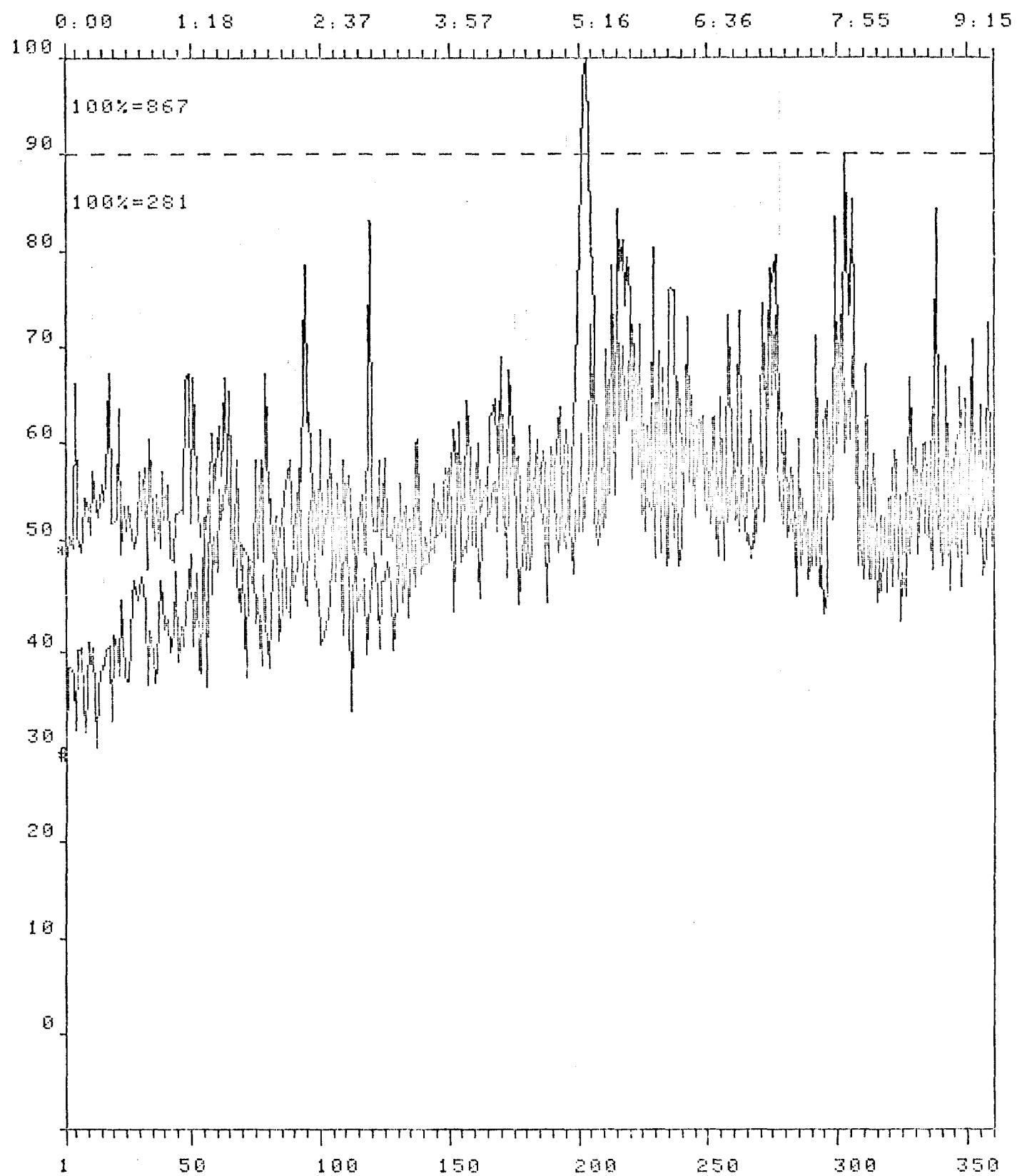
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\* 338 # 340



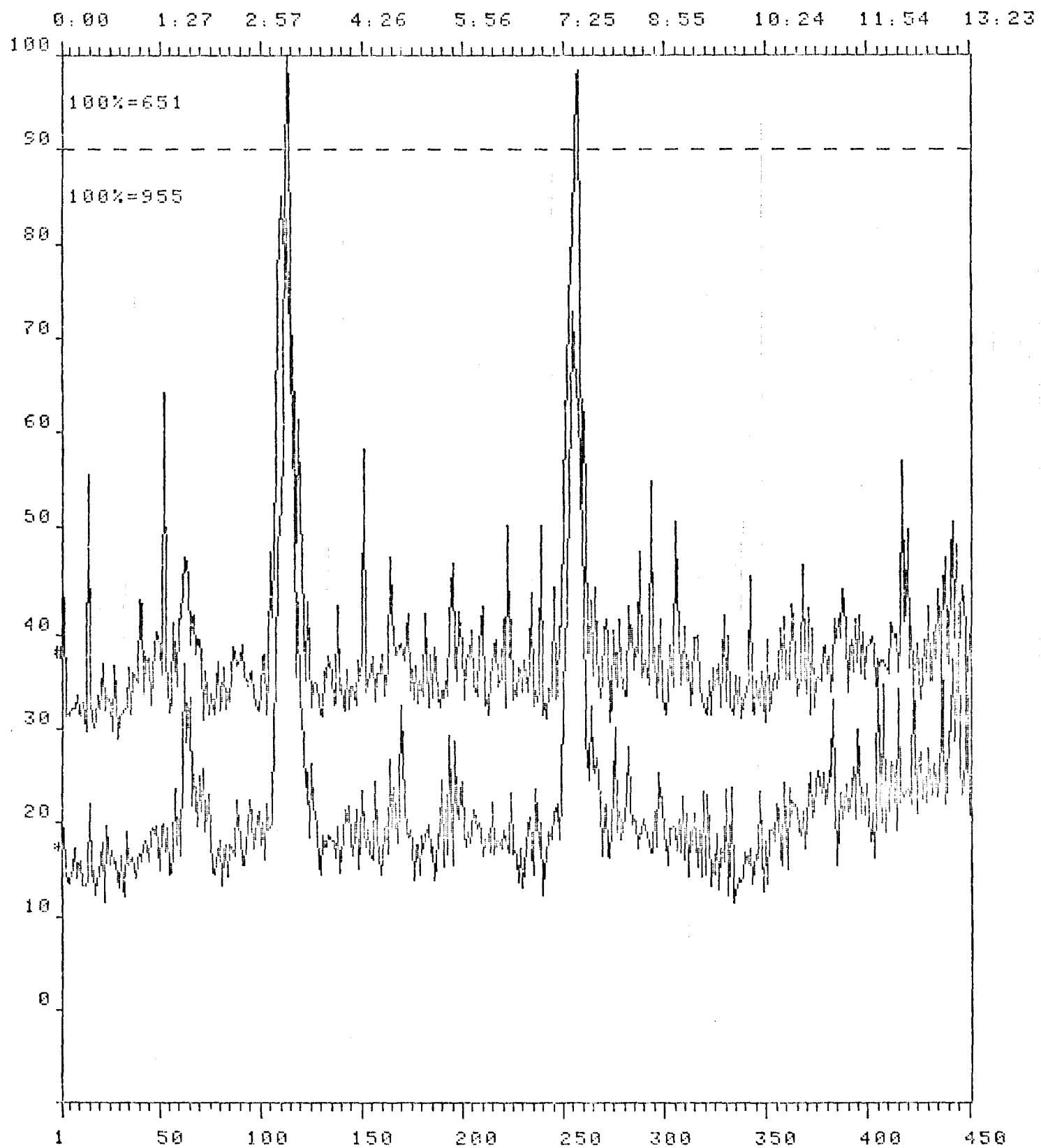
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\* 354 # 356



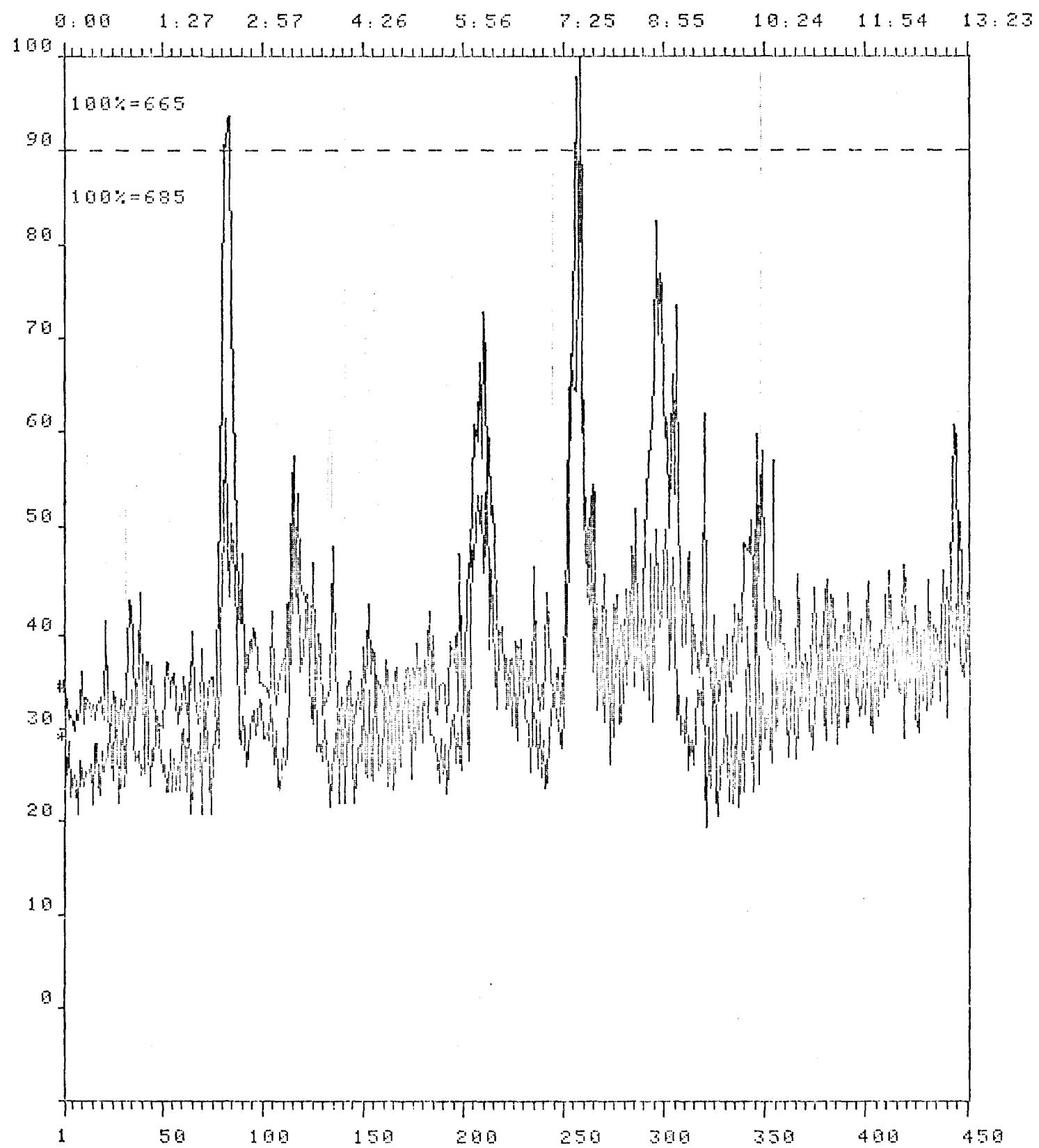
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\* 374 # 376



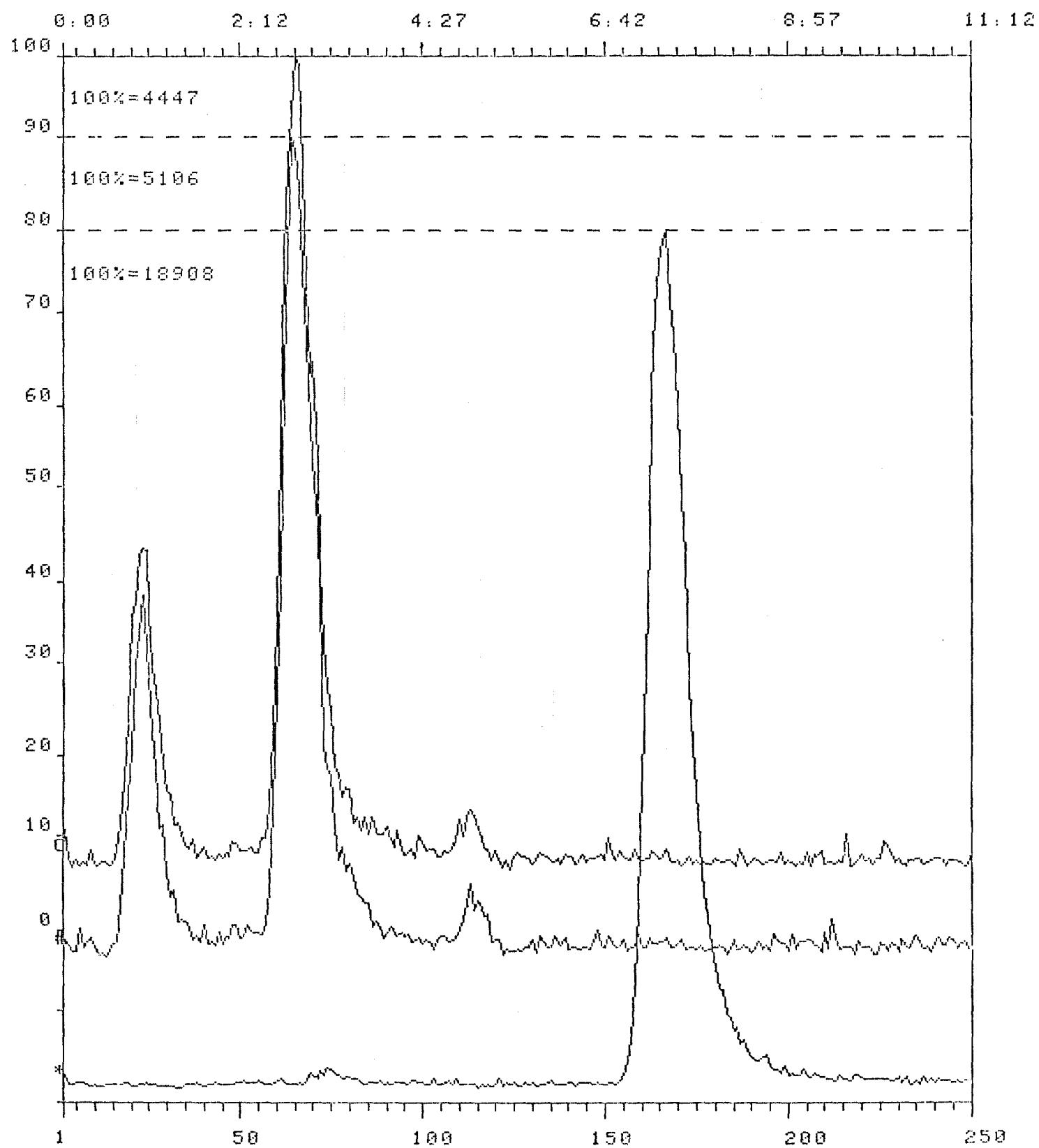
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\* 390 # 392



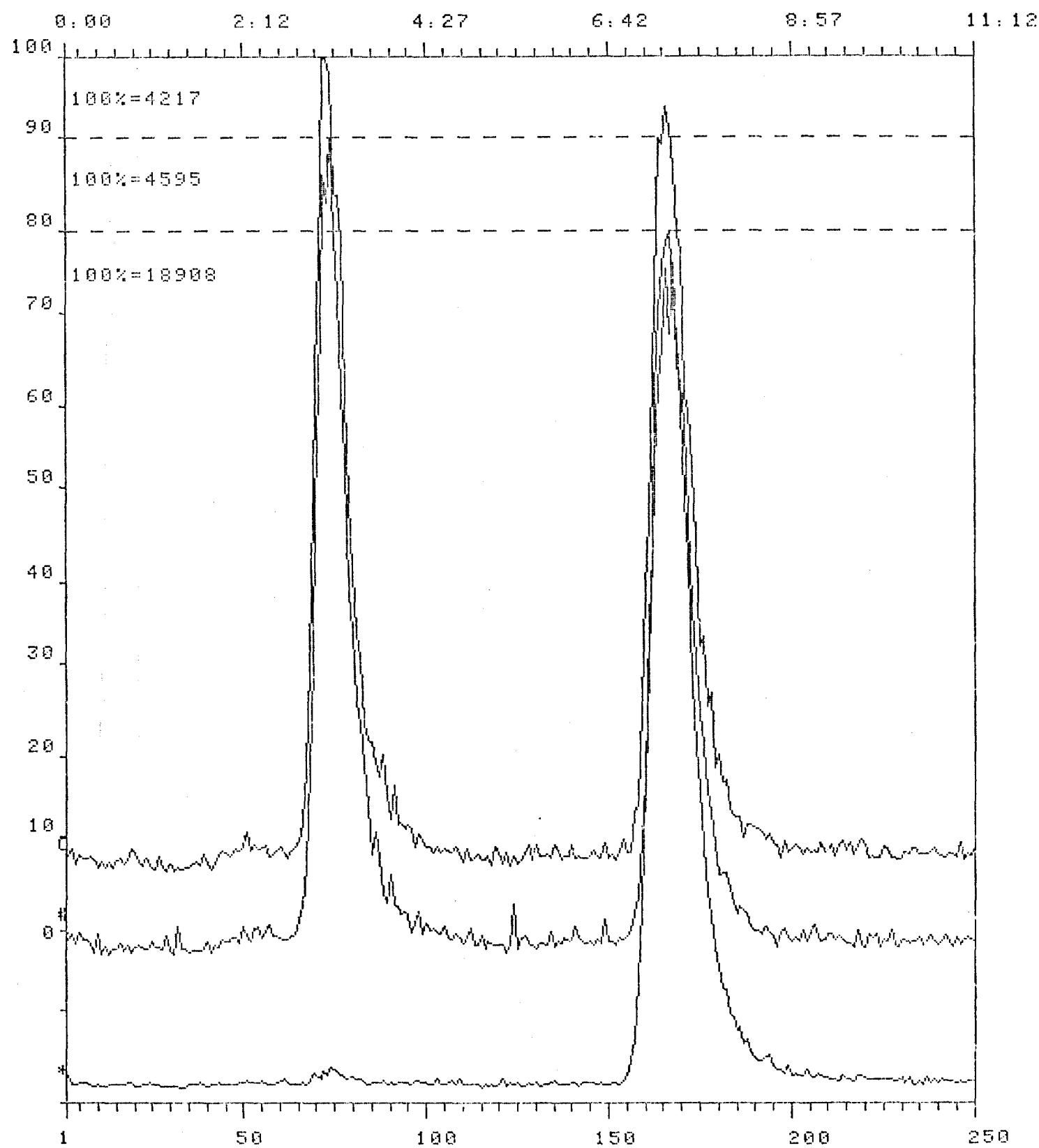
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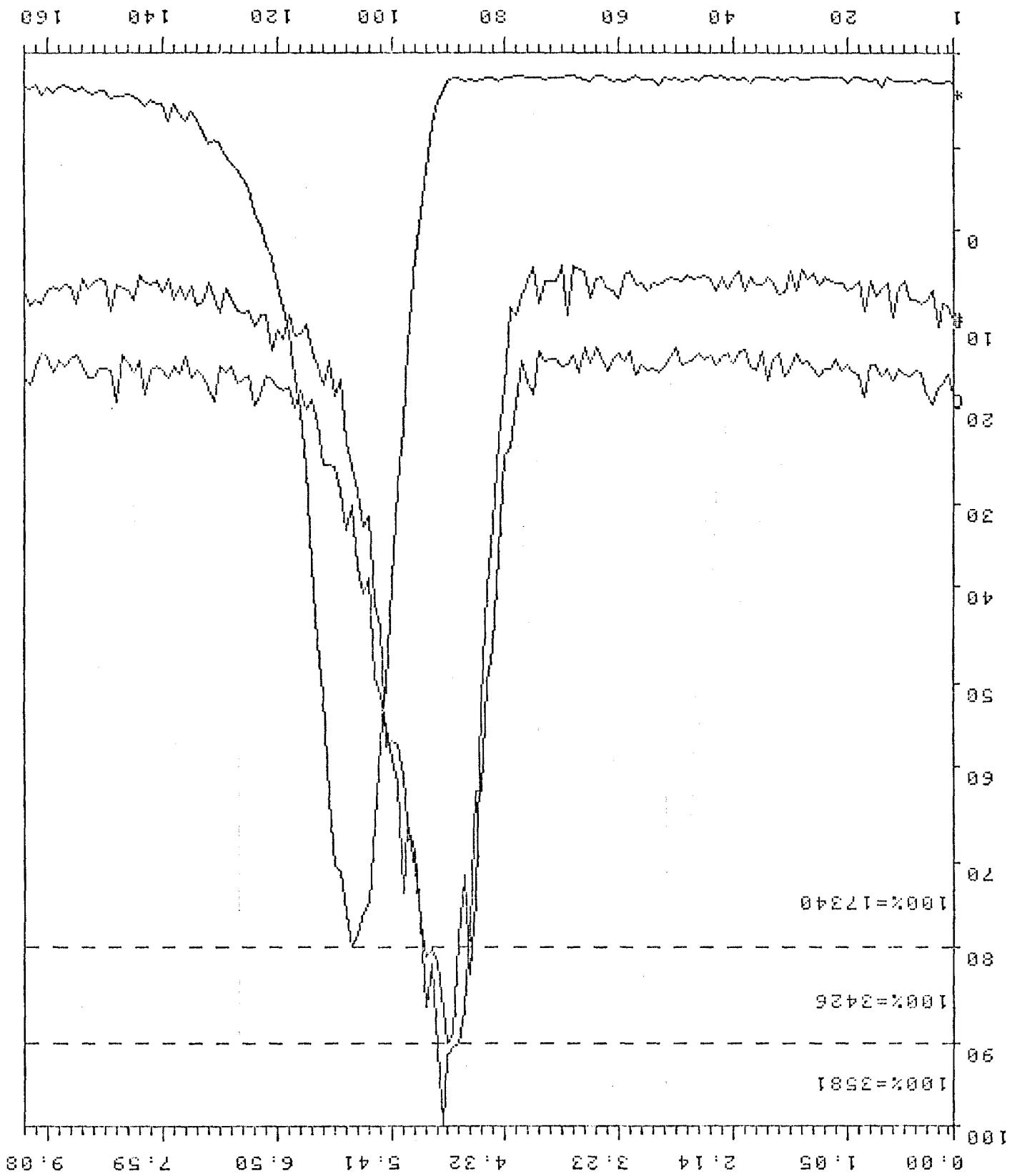
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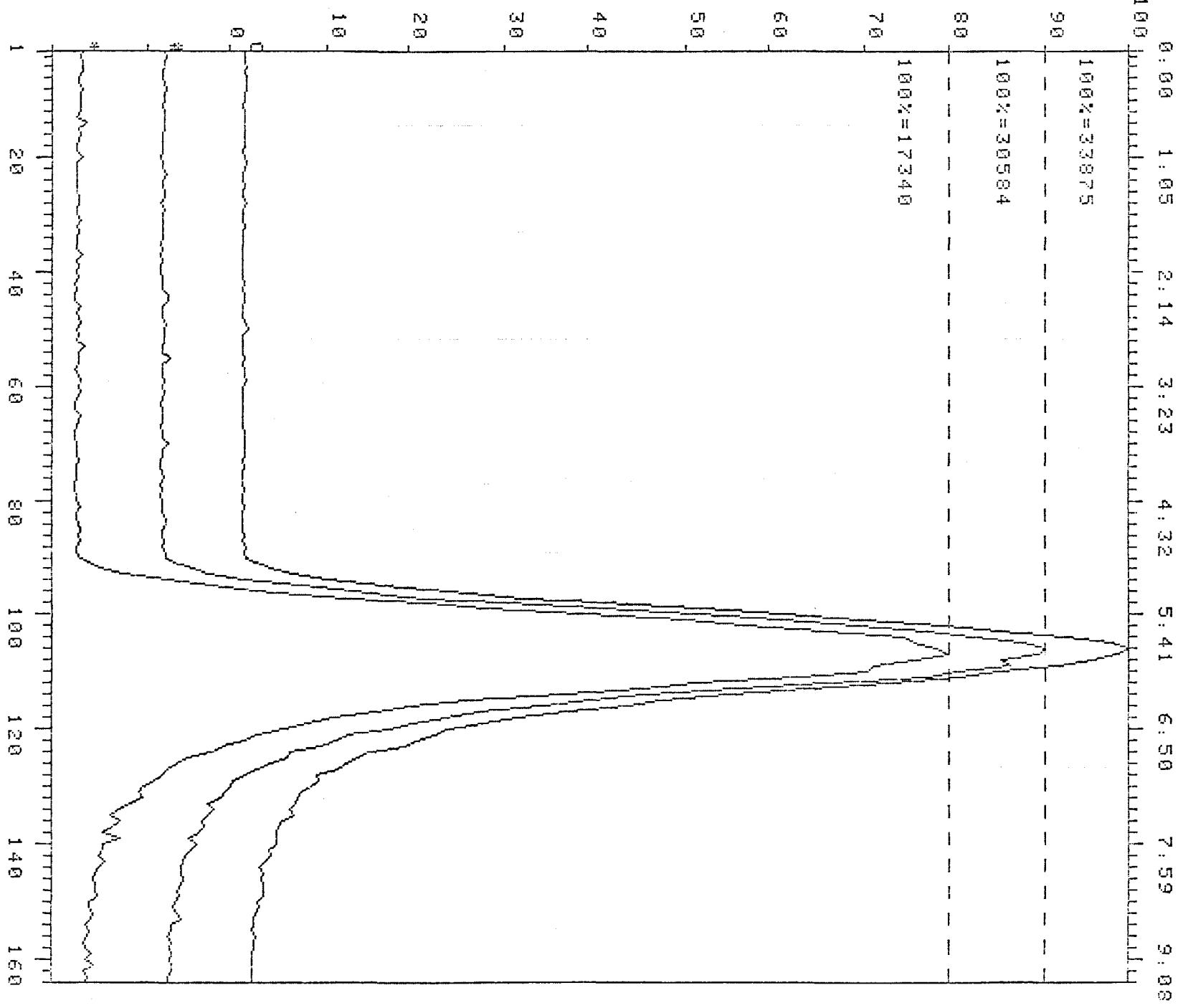




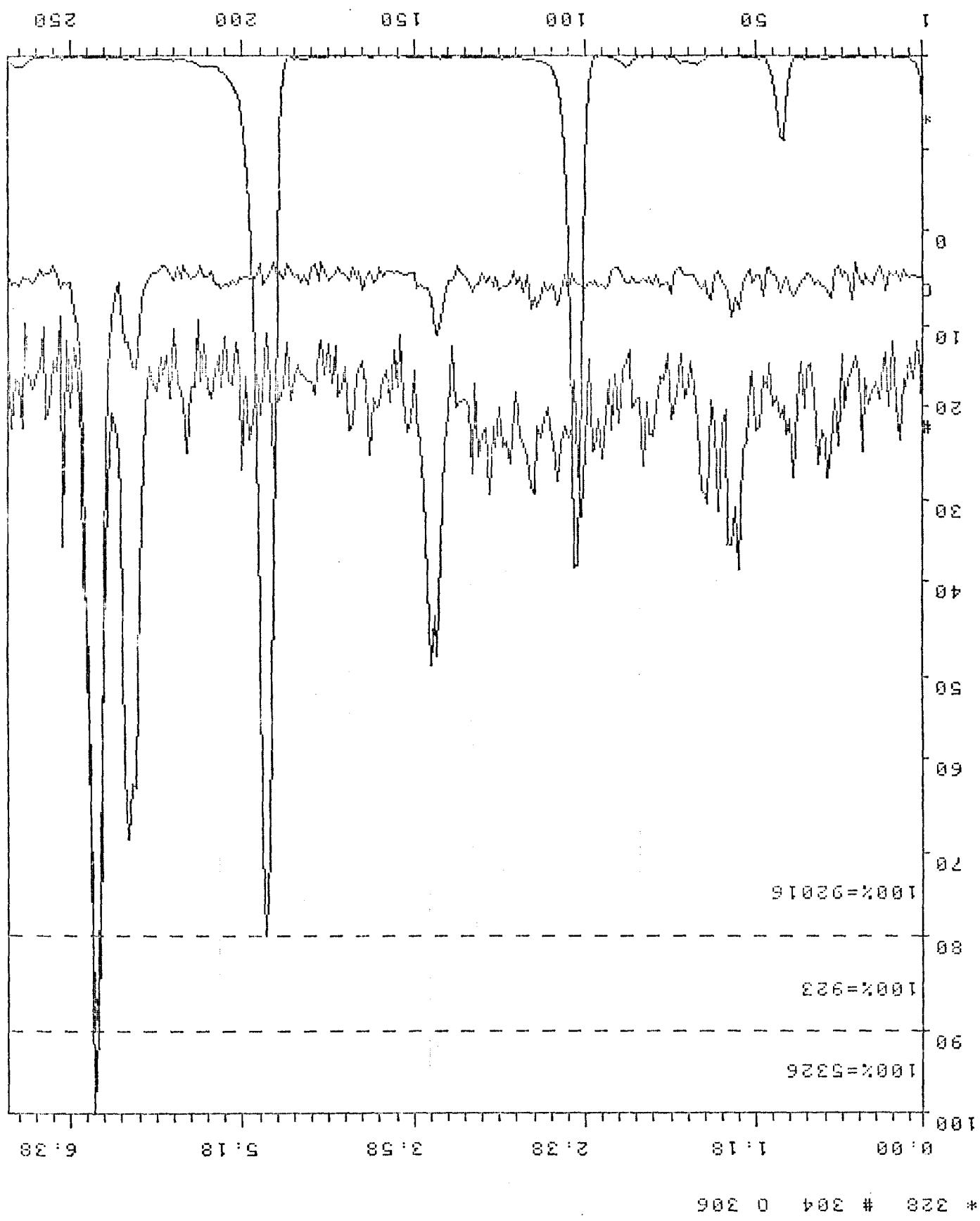
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\* 472 # 458 0 460



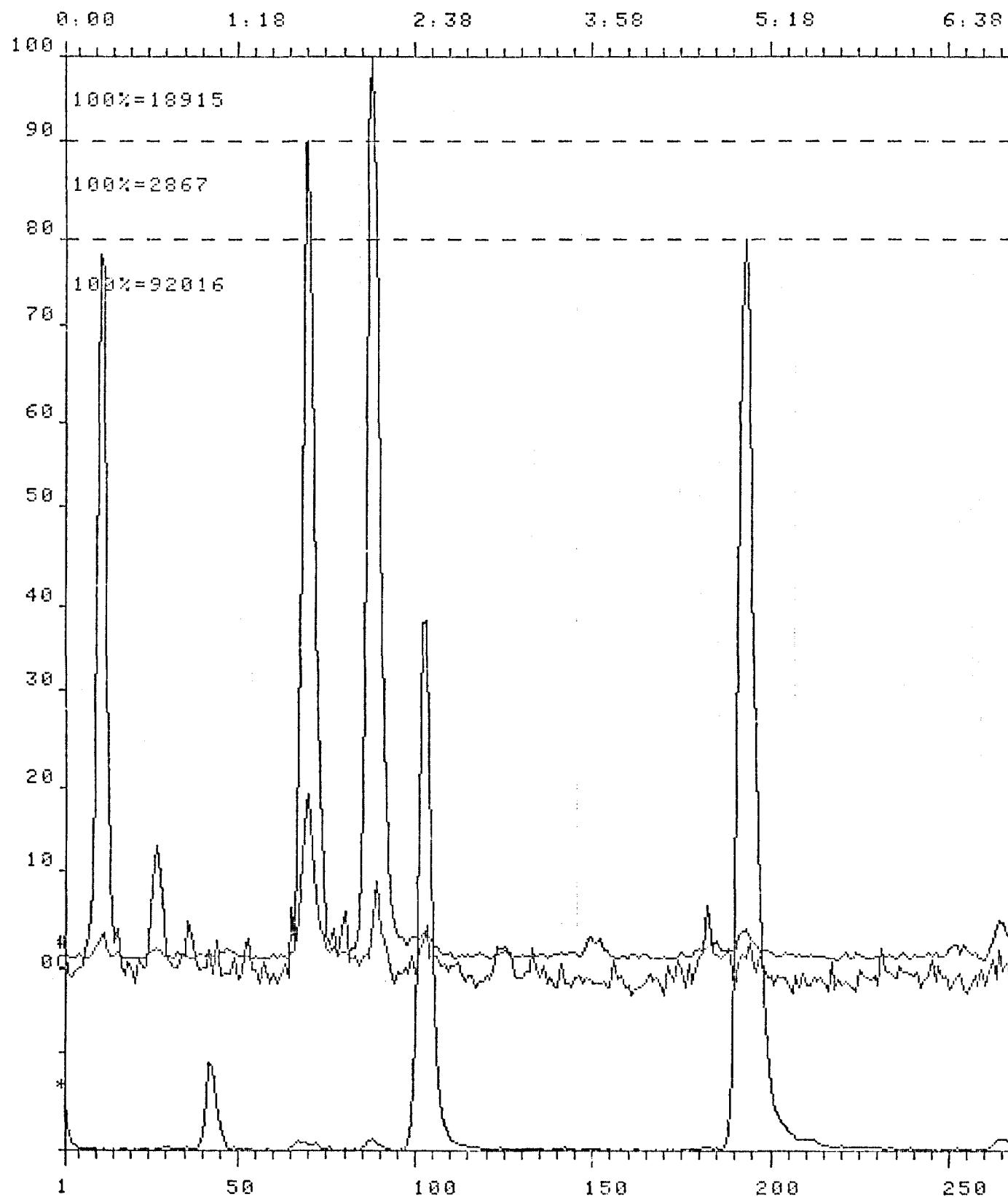
c. CWS-2



\* 328 # 384 O 306 DS-55 CROSS SCAN REPORT, RUN, CUSB40001

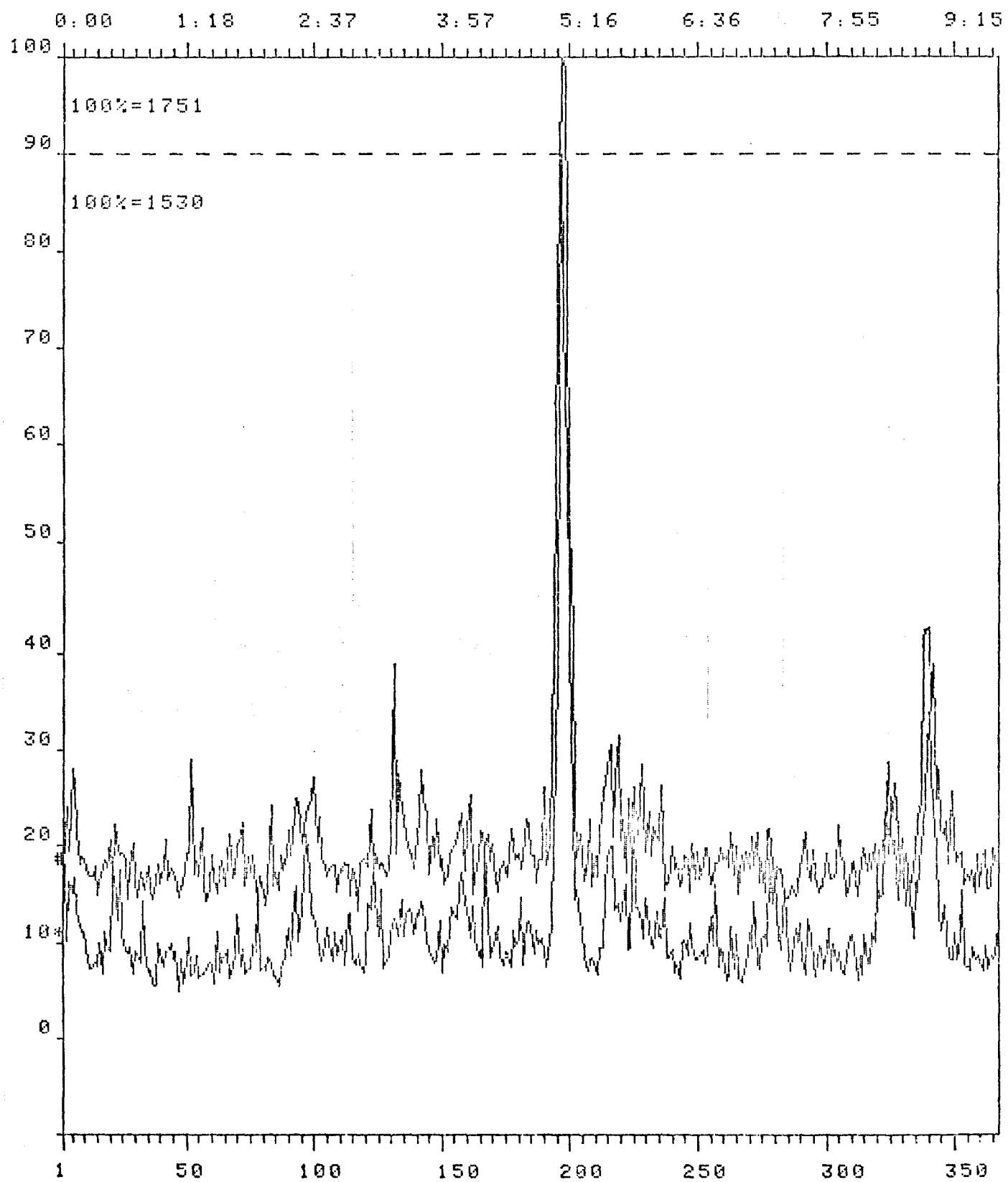
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\* 328 # 320 O 322



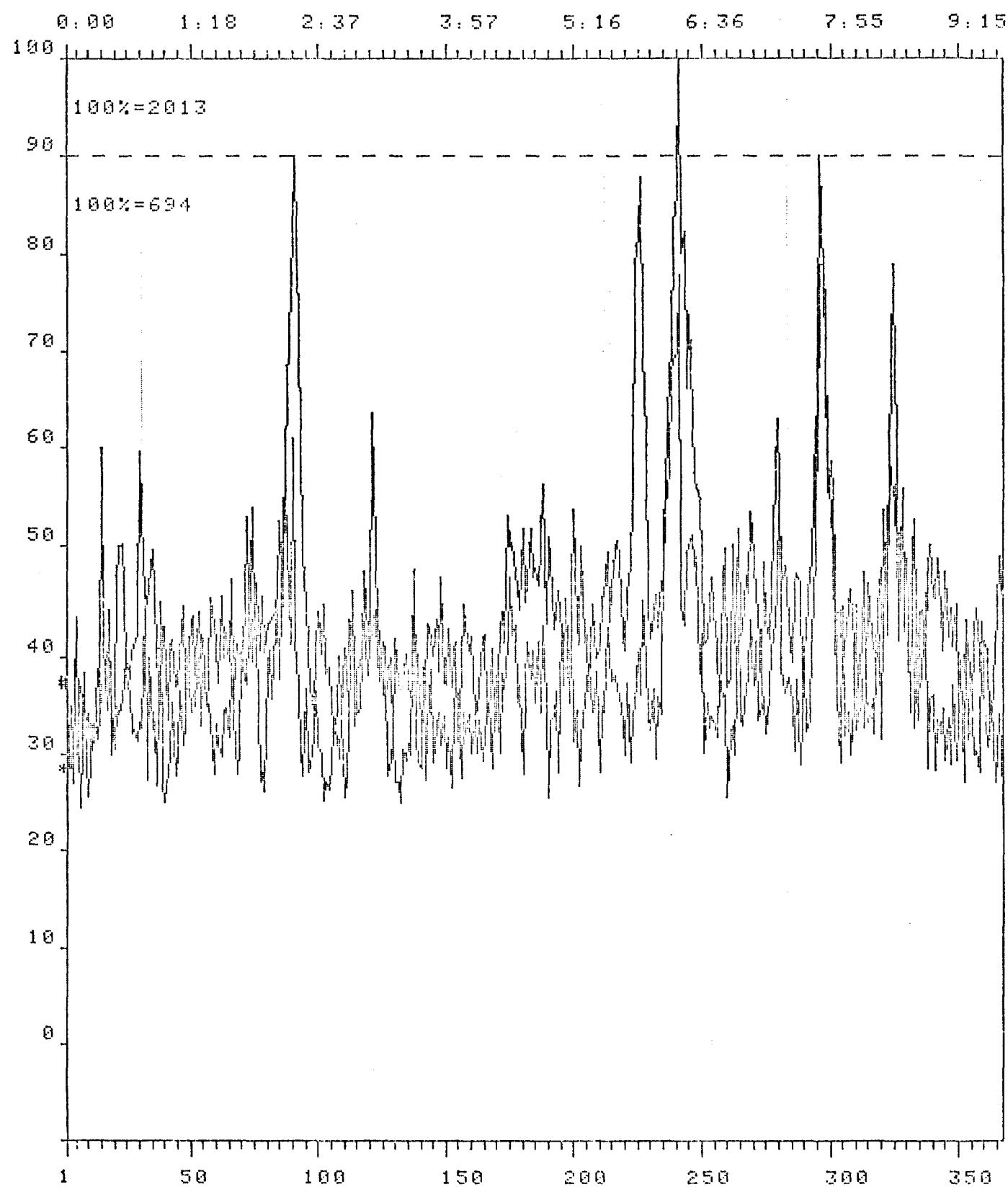
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\* 338 # 340



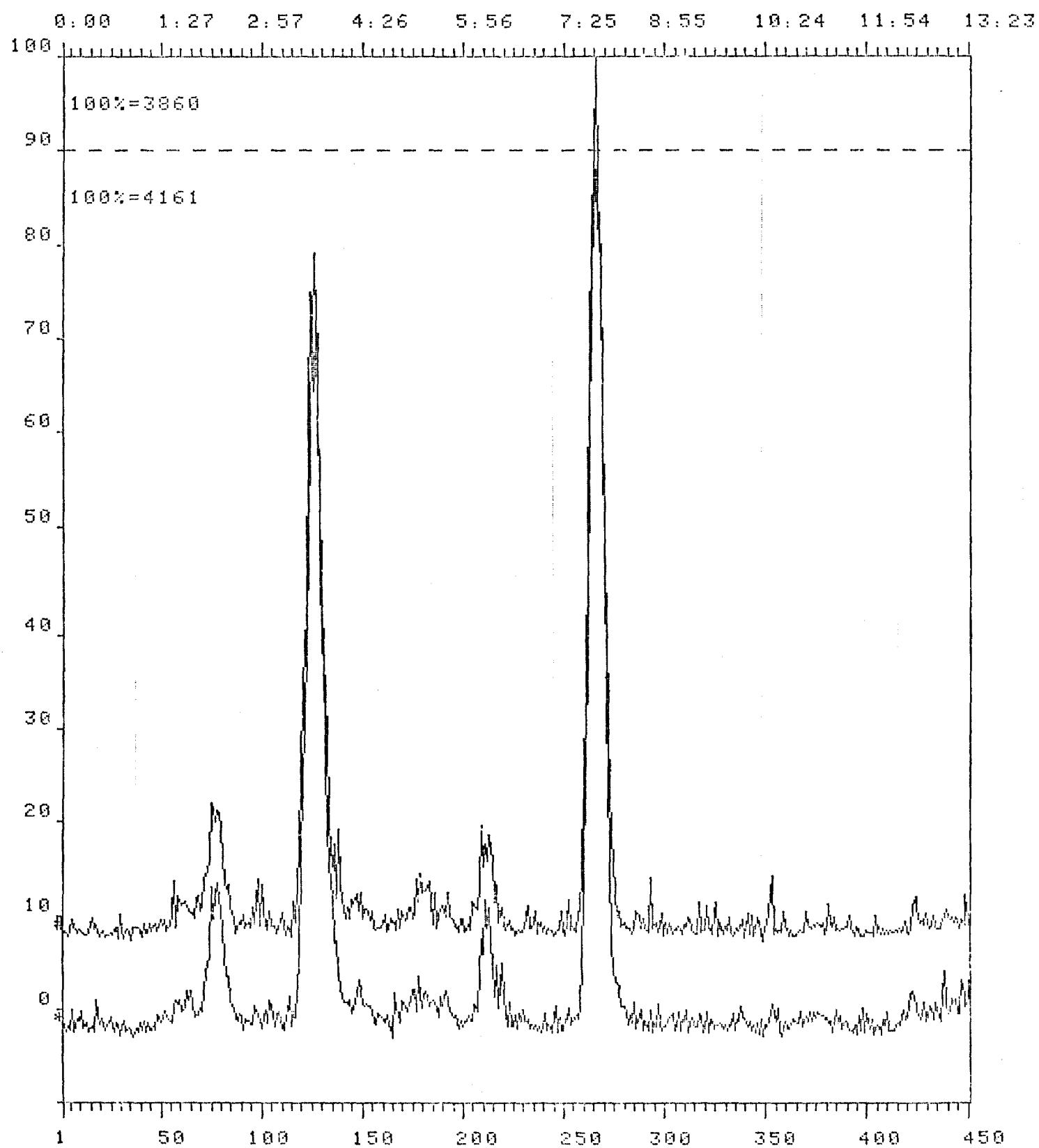
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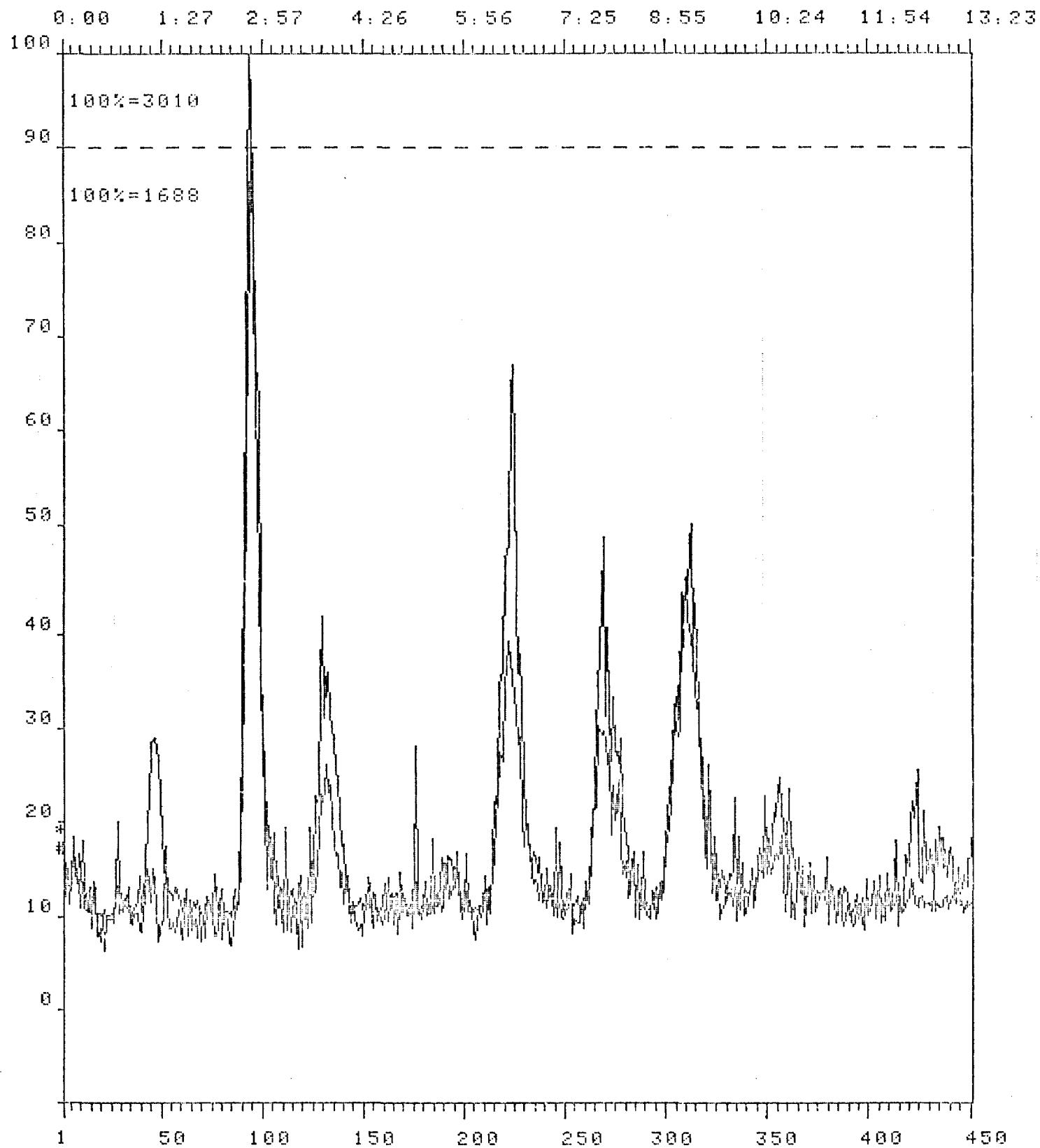
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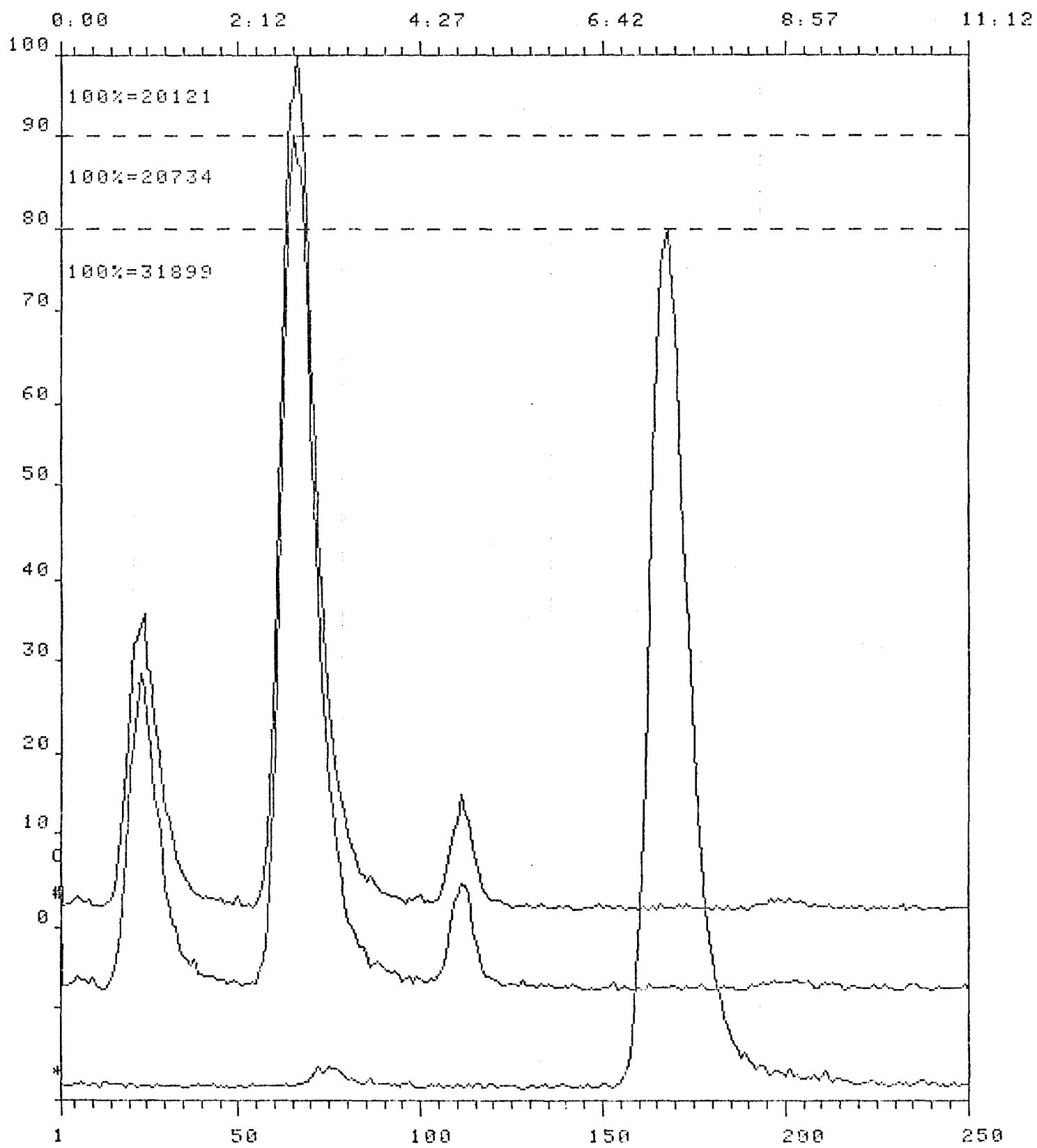
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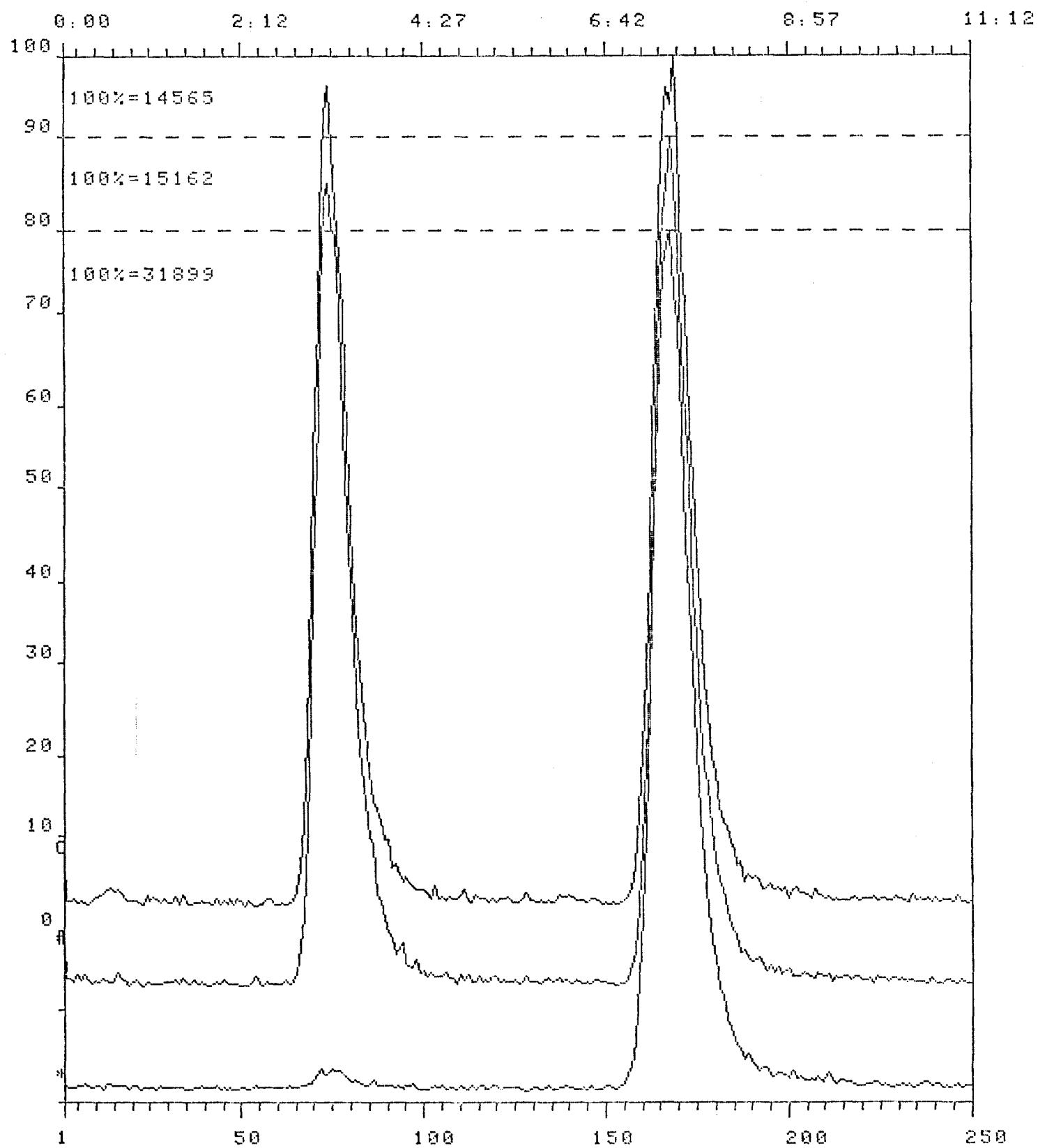
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\* 432 # 408 O 410



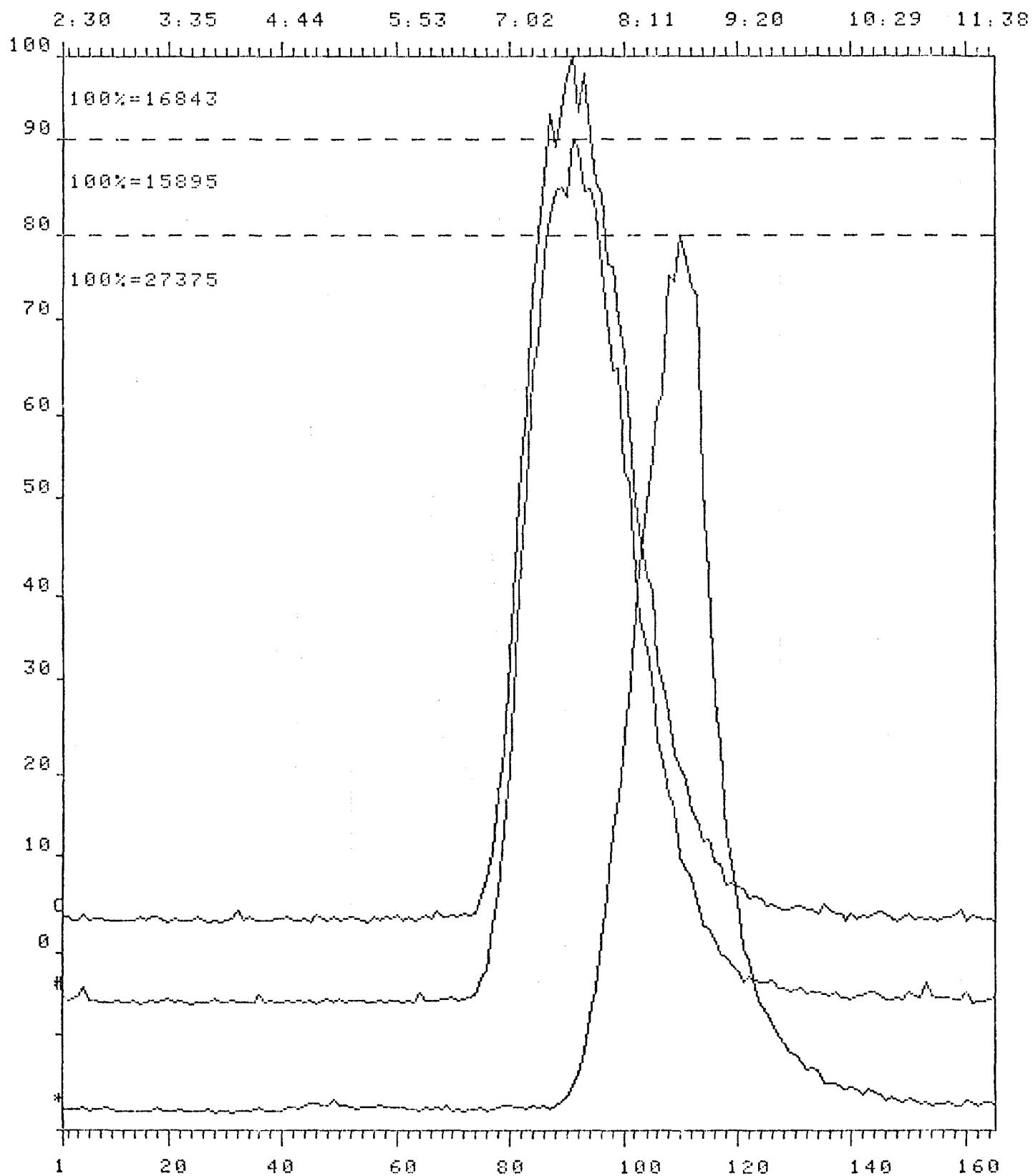
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\* 432 # 424 O 426



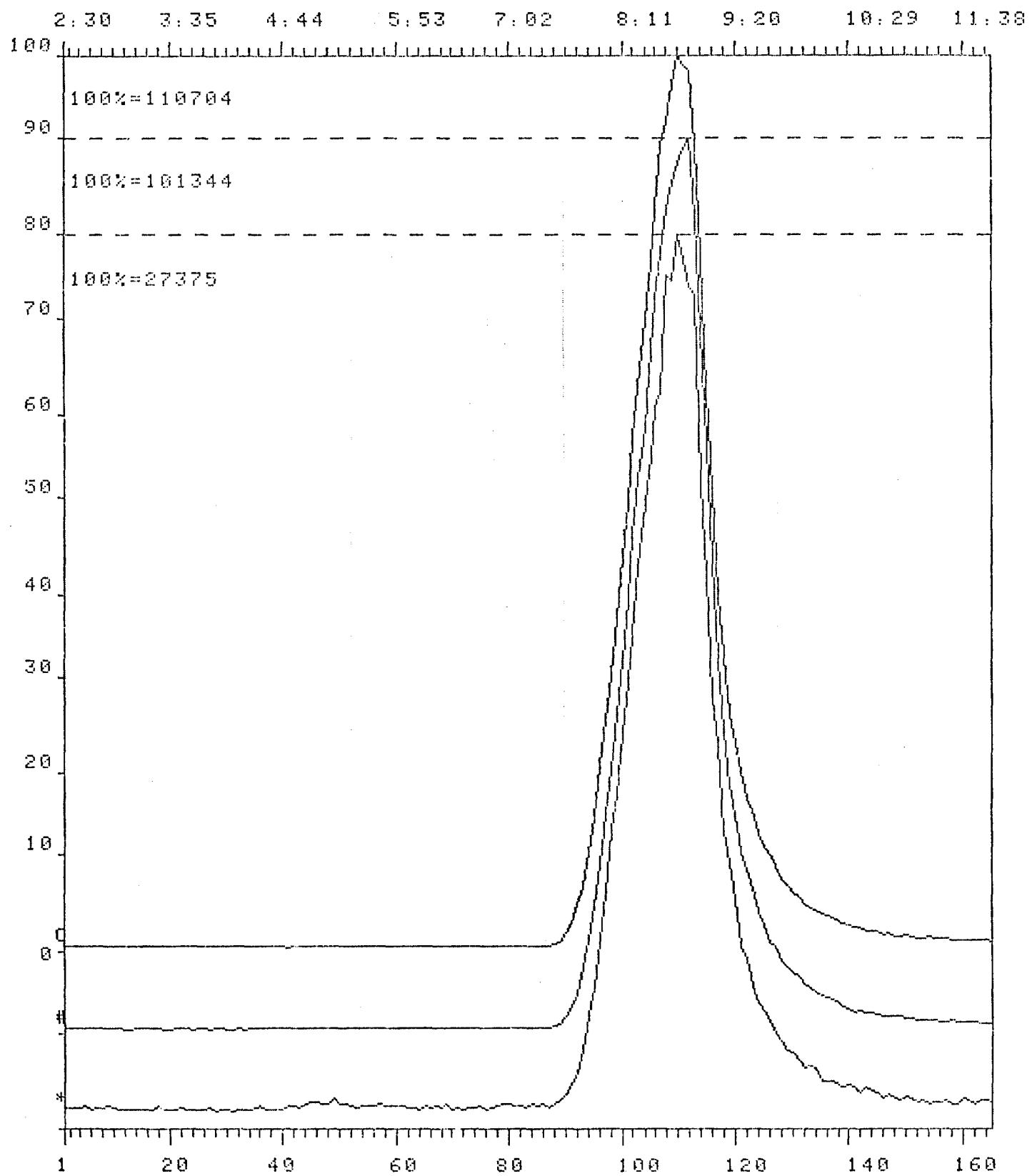
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\* 472 # 442 O 444

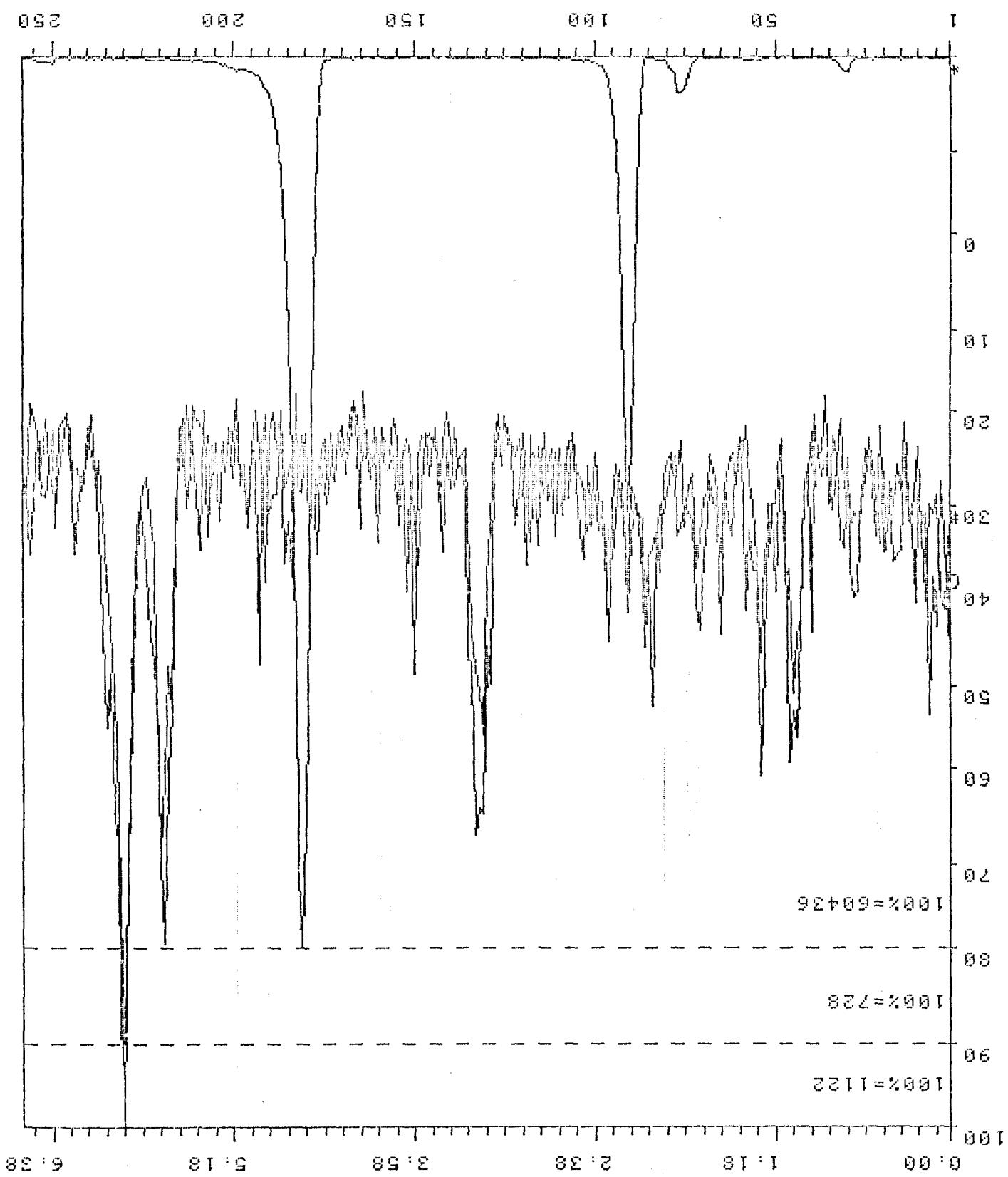


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\* 472 # 458 O 460



D. CWS-3



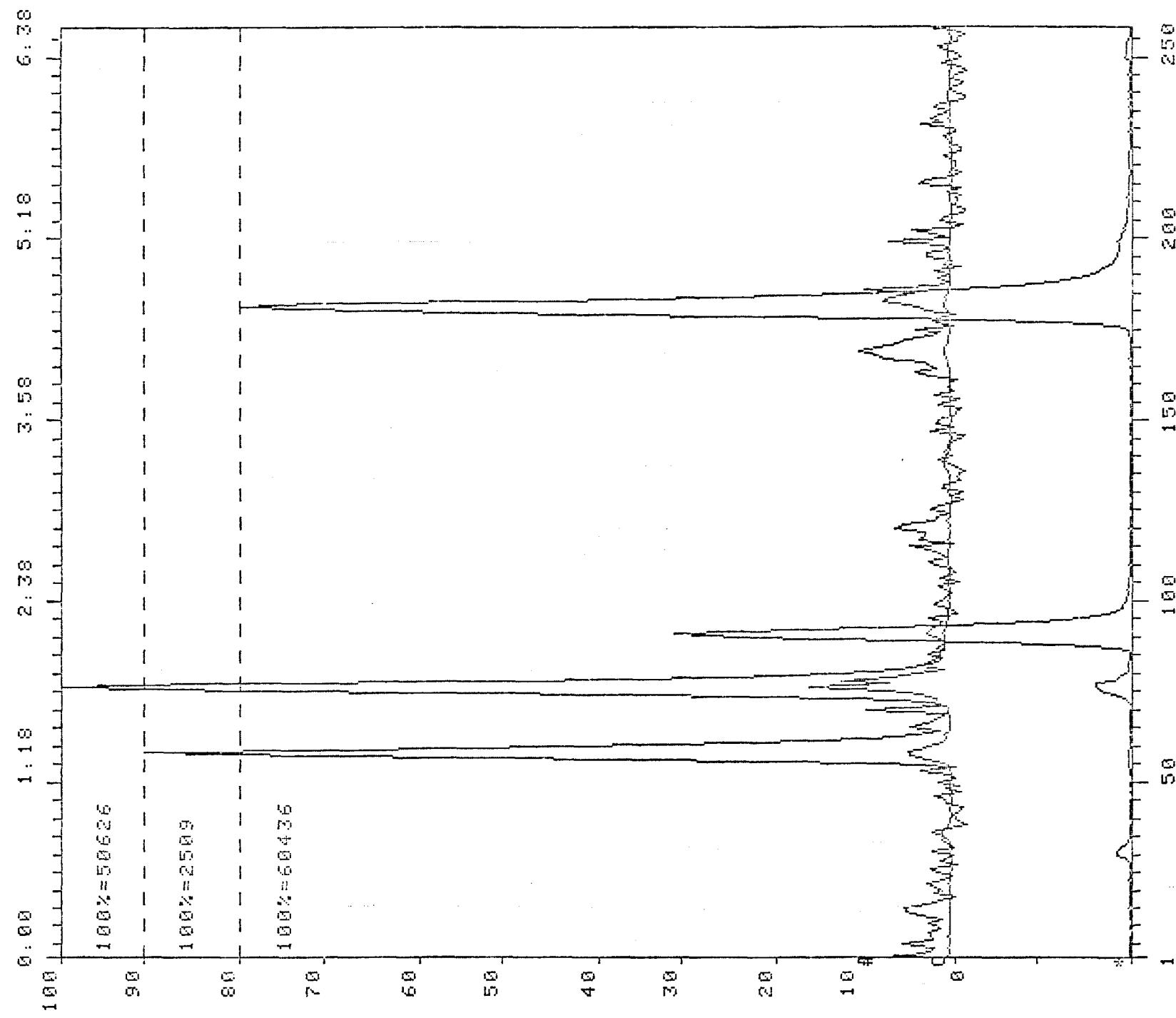
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CWS-5

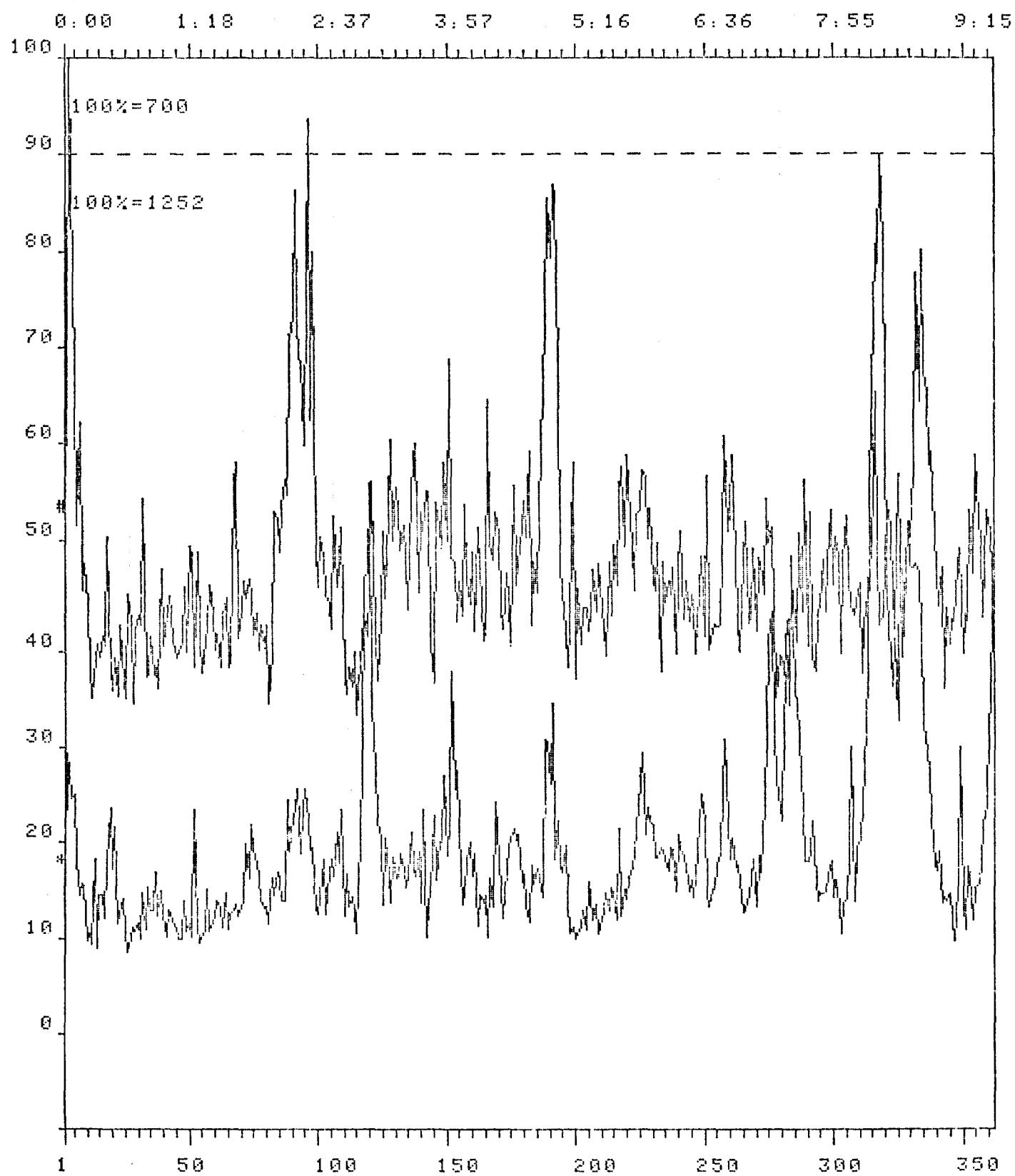
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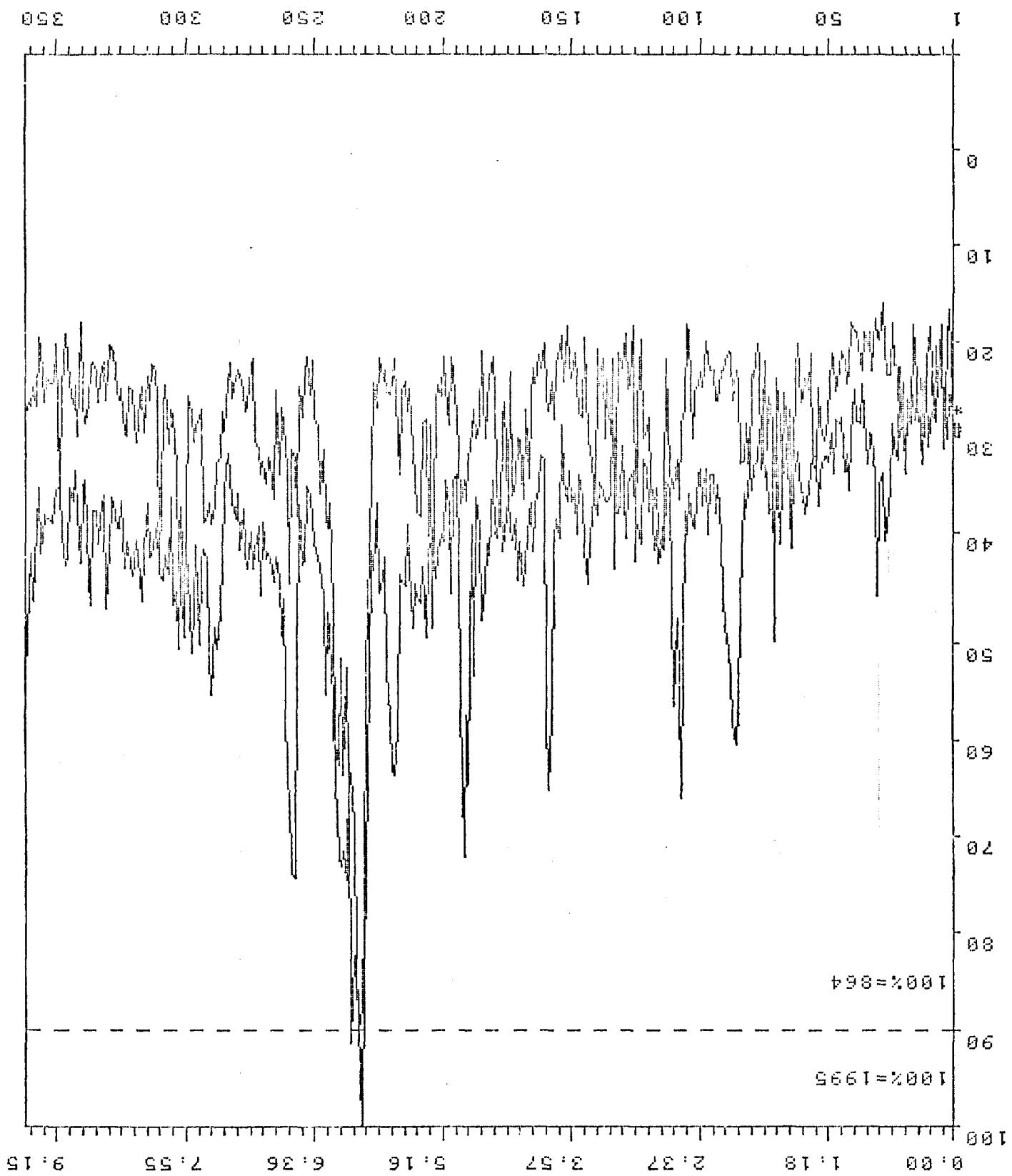
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\* 338 # 340



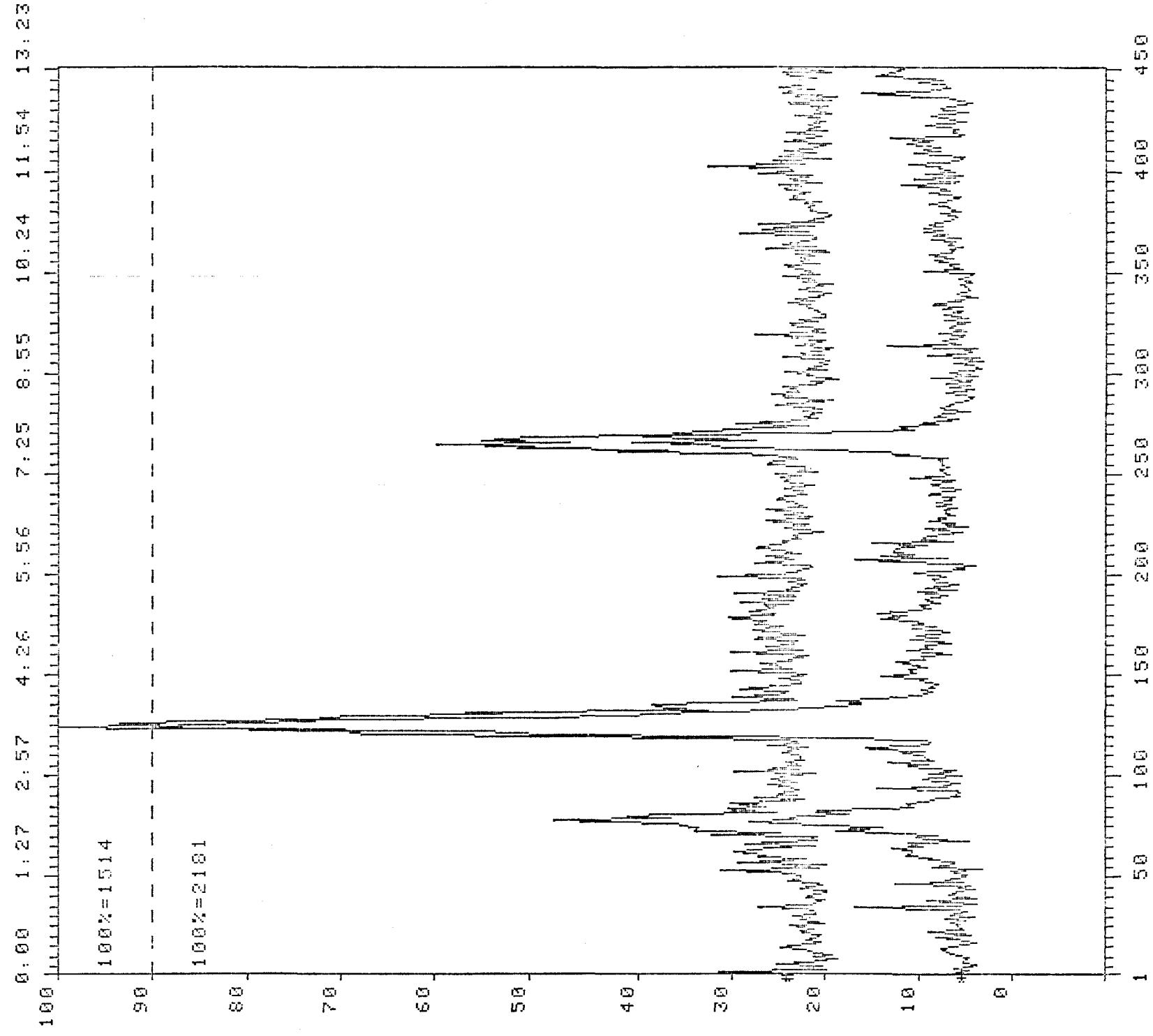


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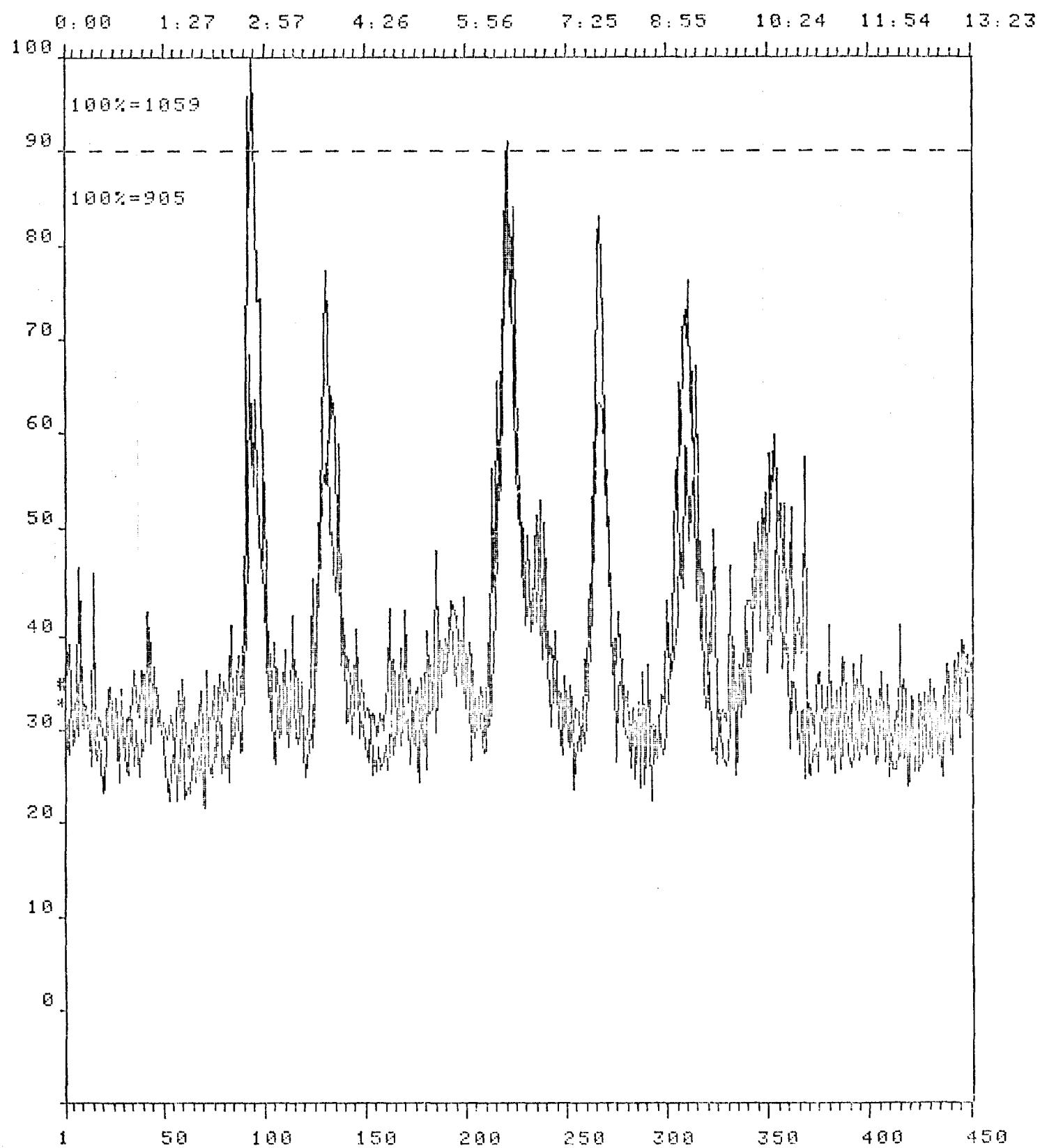
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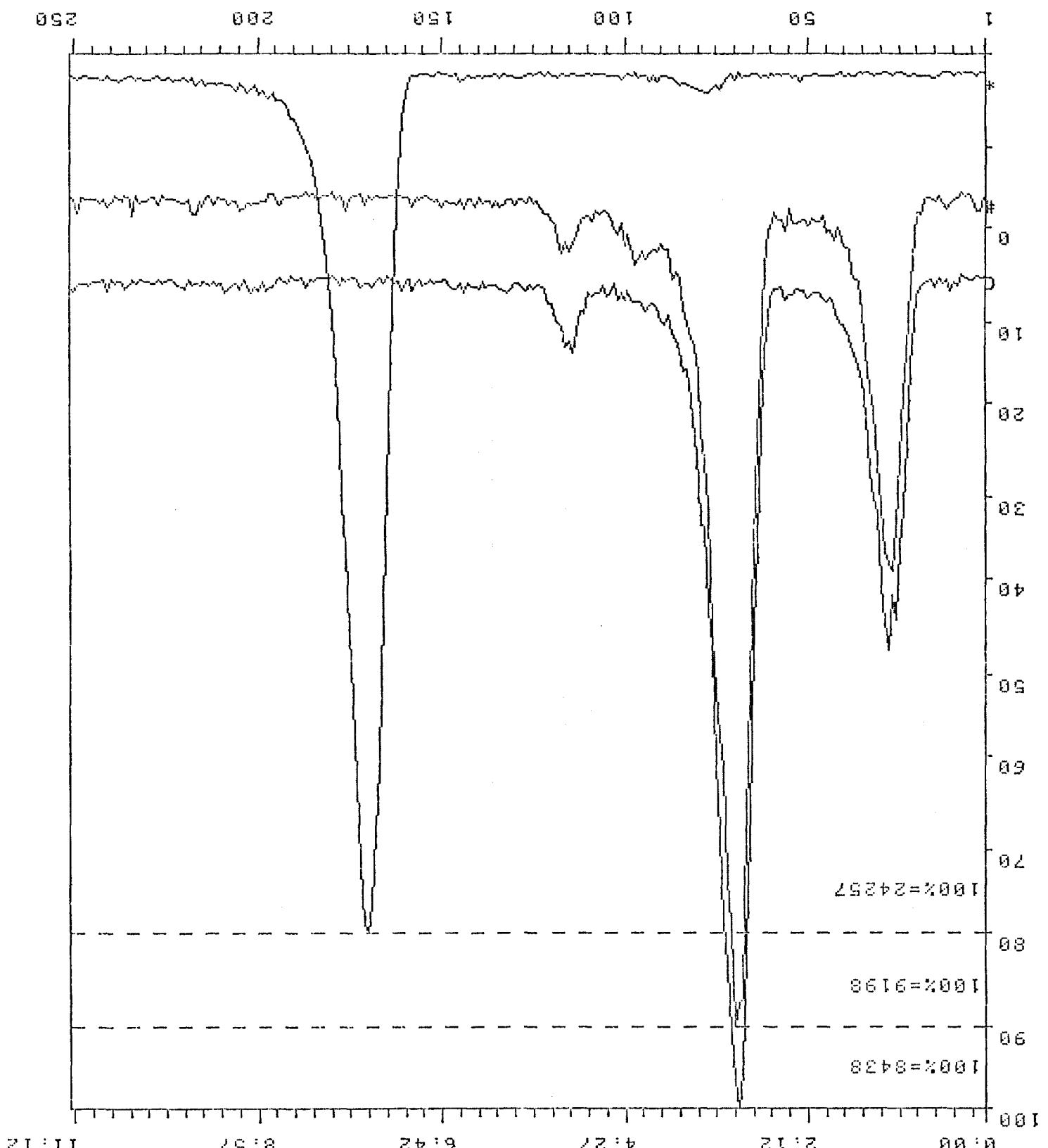
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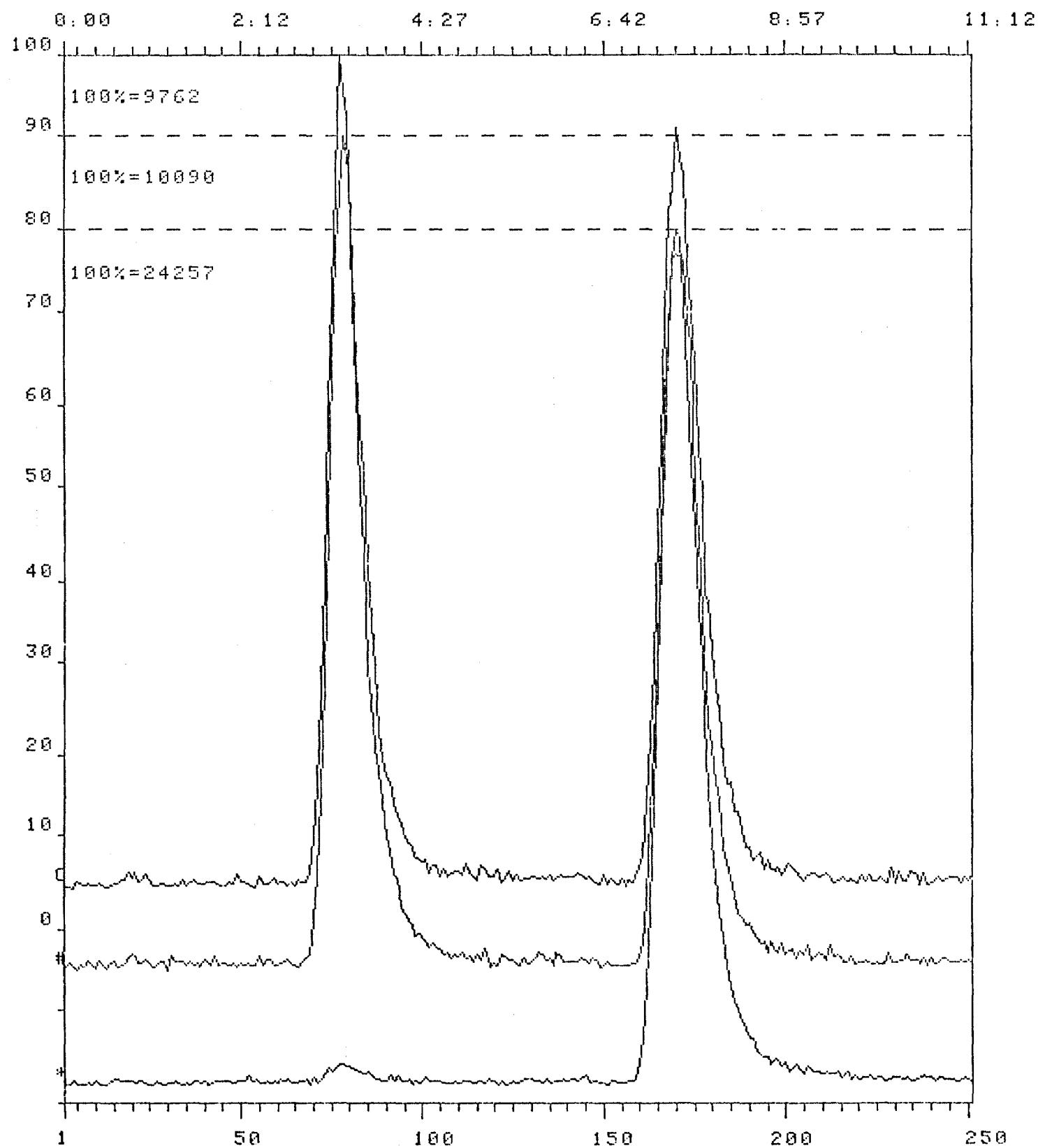
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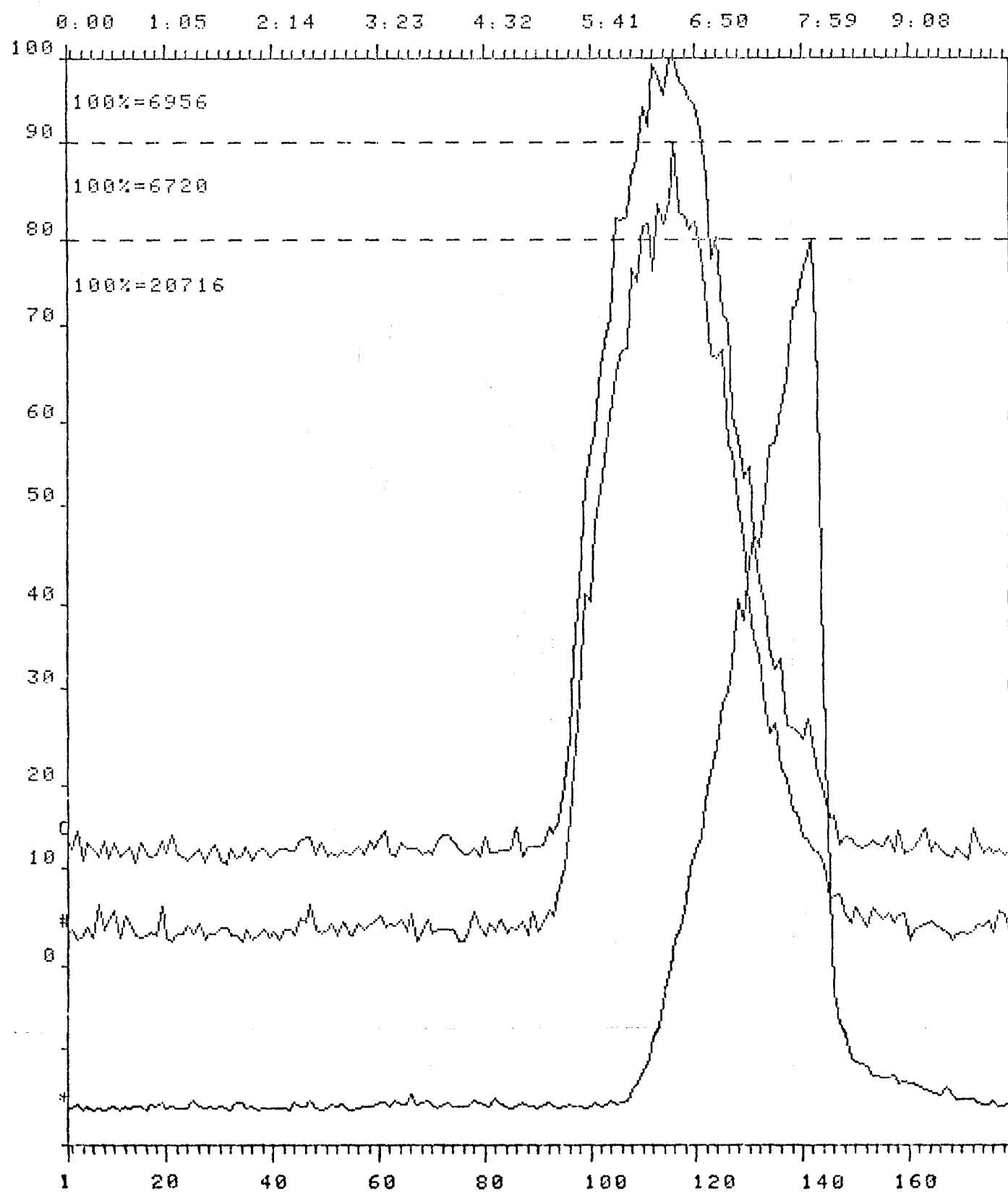
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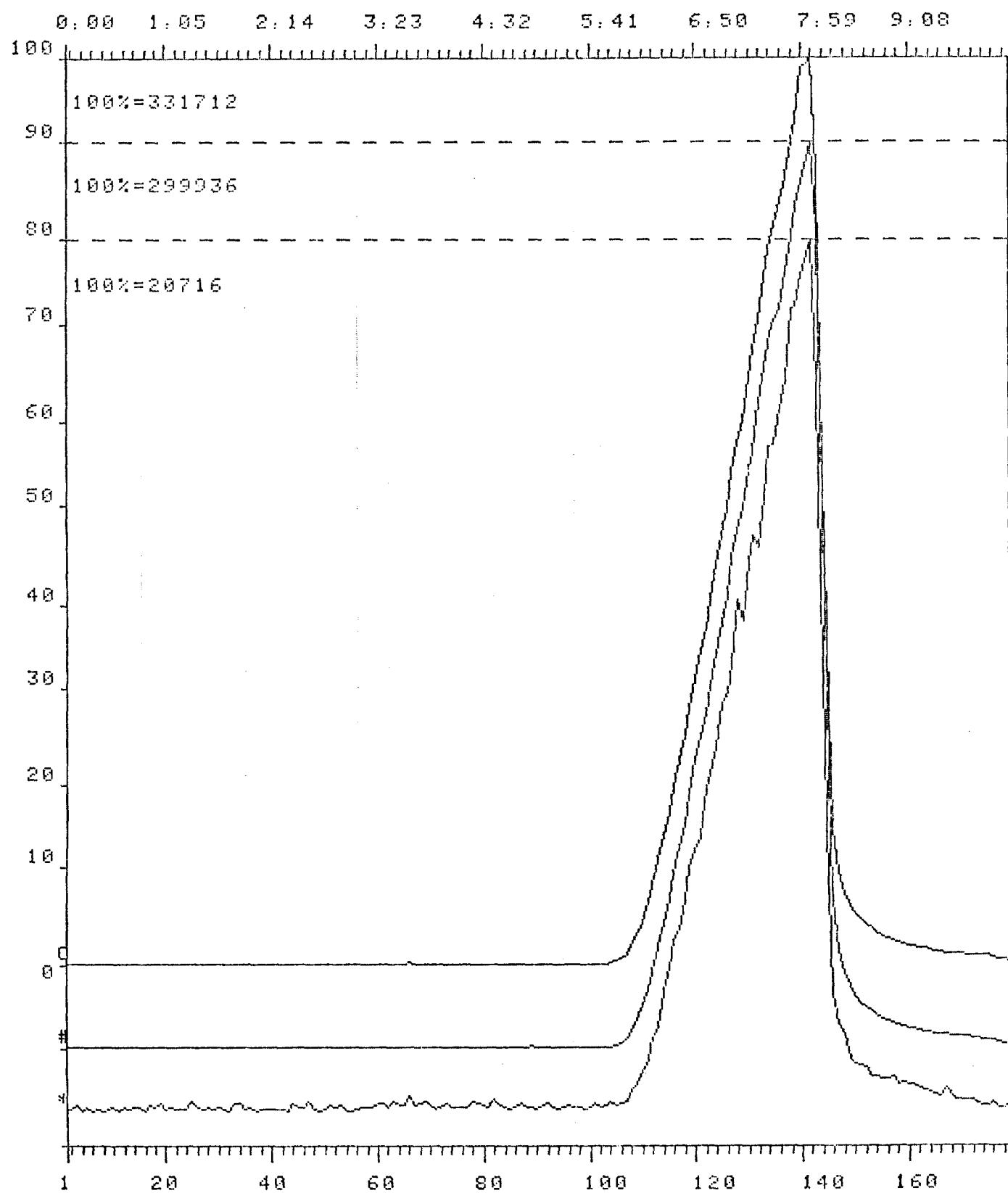
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\* 472 # 442 O 444



DS-55 CROSS SCAN REPORT, RUN: CWS80006

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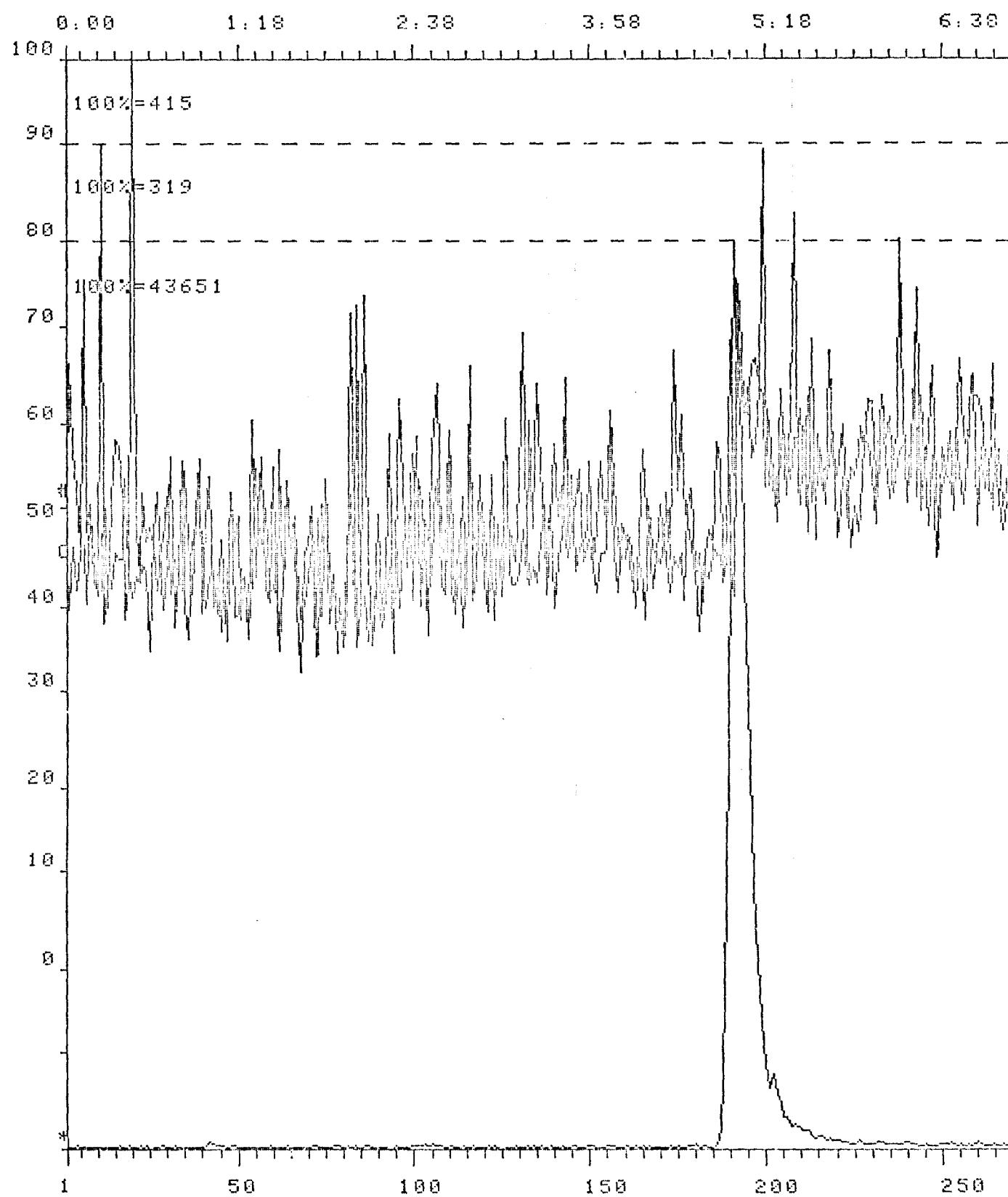


E. CMS-4

CW 5-7

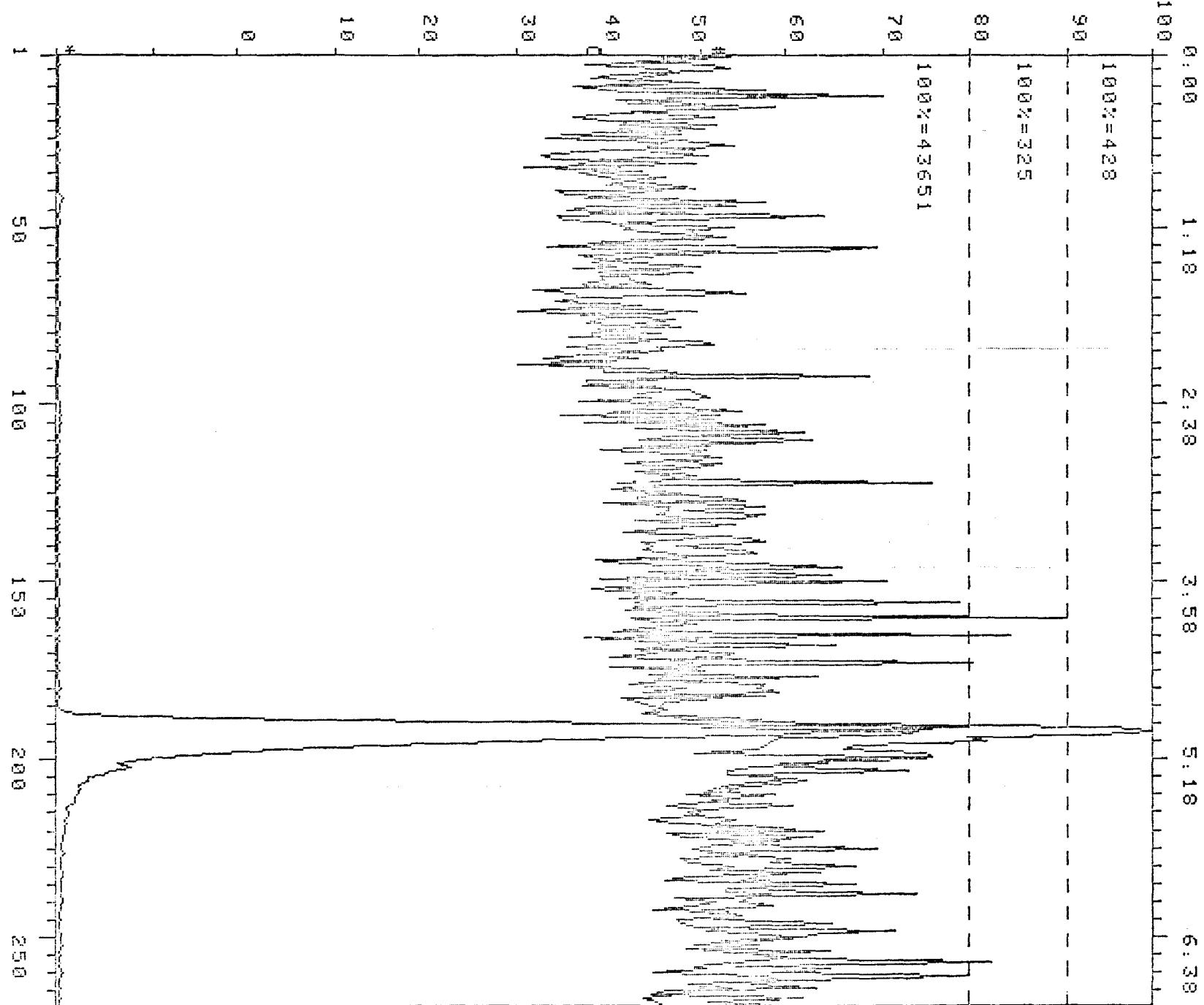
DS-55 CROSS SCAN REPORT, RUN: CWSB40003

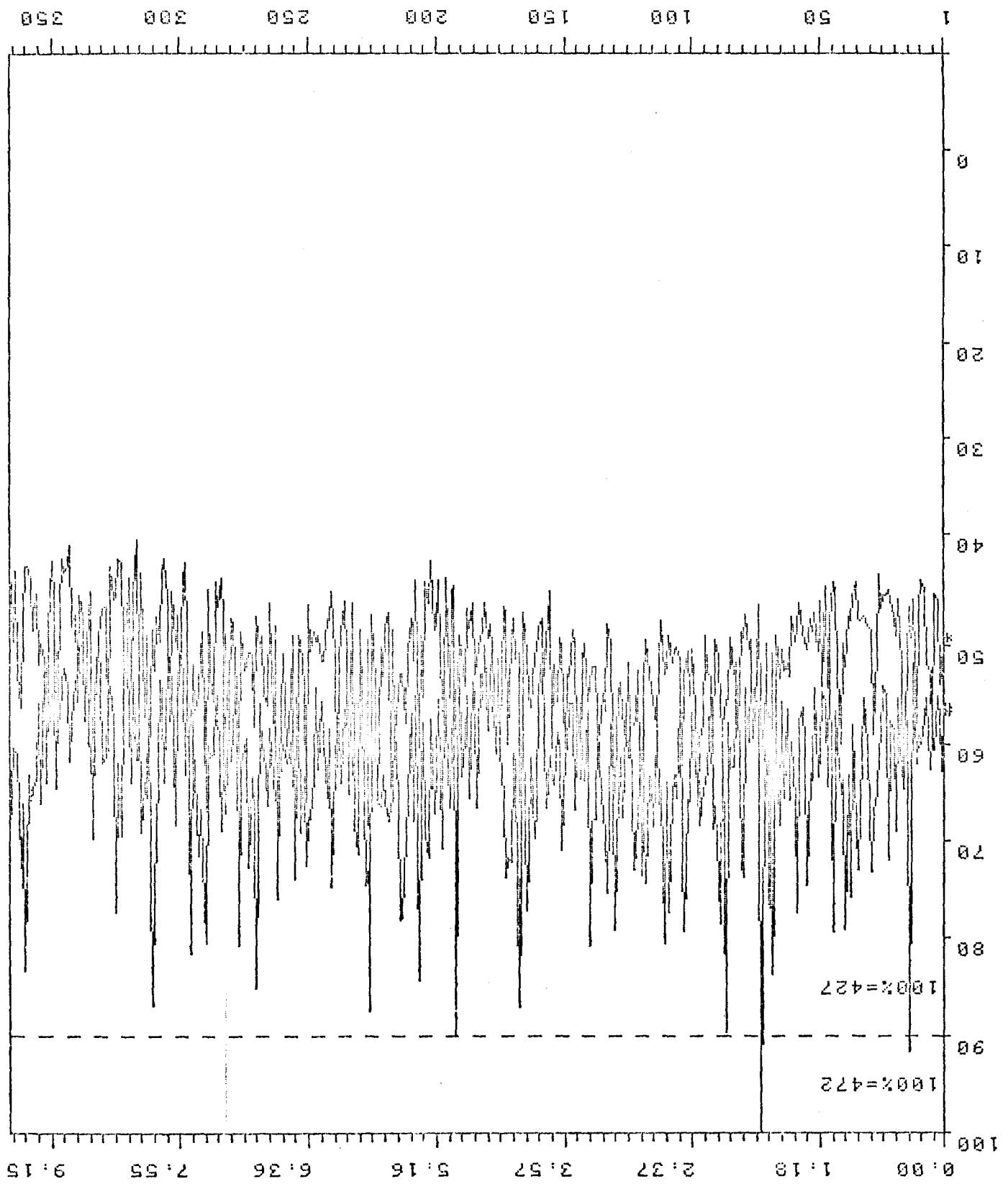
\* 328 # 304 0 306



DS-55 CROSS SCAN REPORT, RUN: CMSB40003

\* 328 # 326 0 322

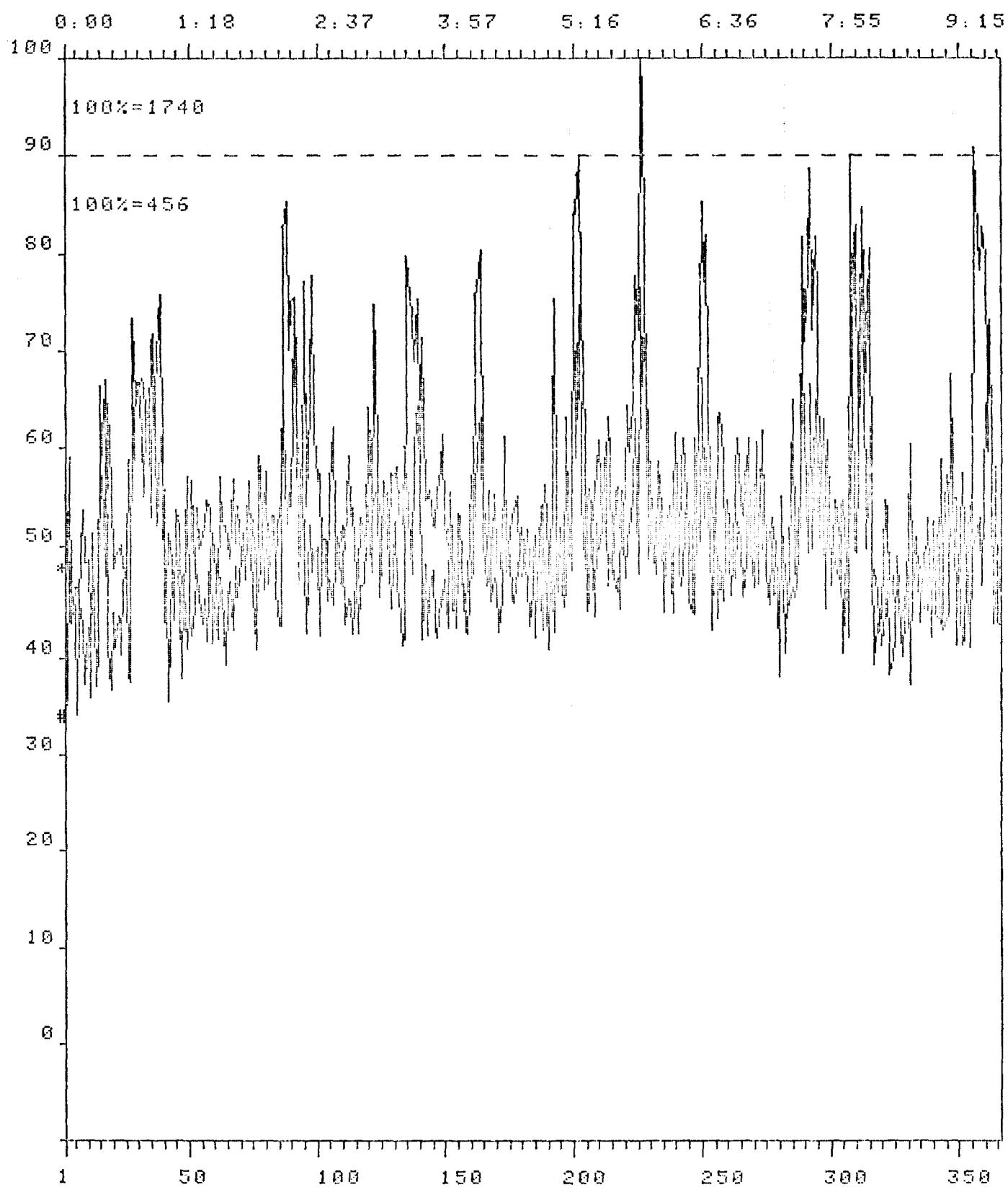




DS-55 CROSS SCAN REPORT, RUH, CW558005

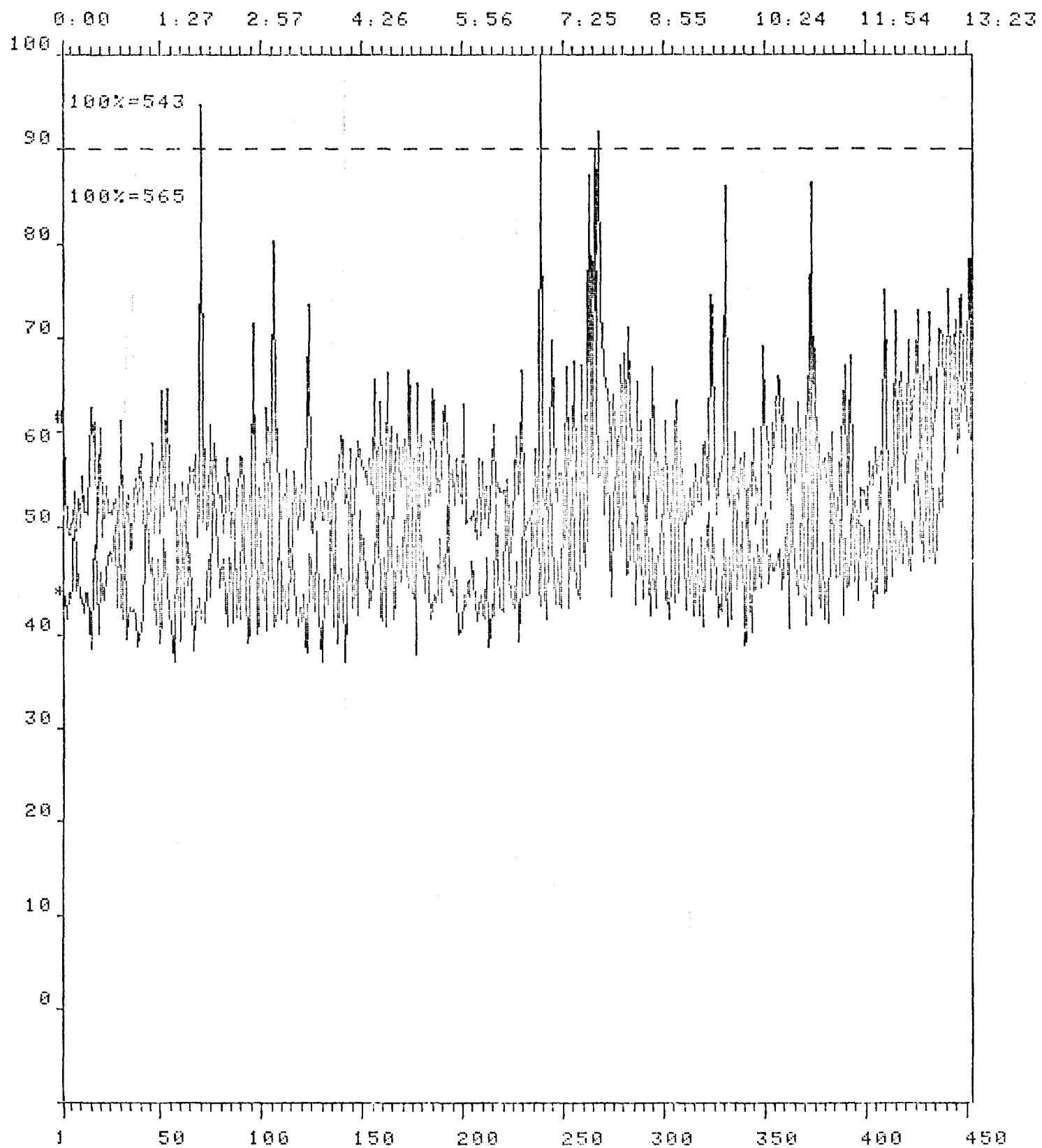
DS-55 CROSS SCAN REPORT, RUN: CWS50005

\* 354 # 356



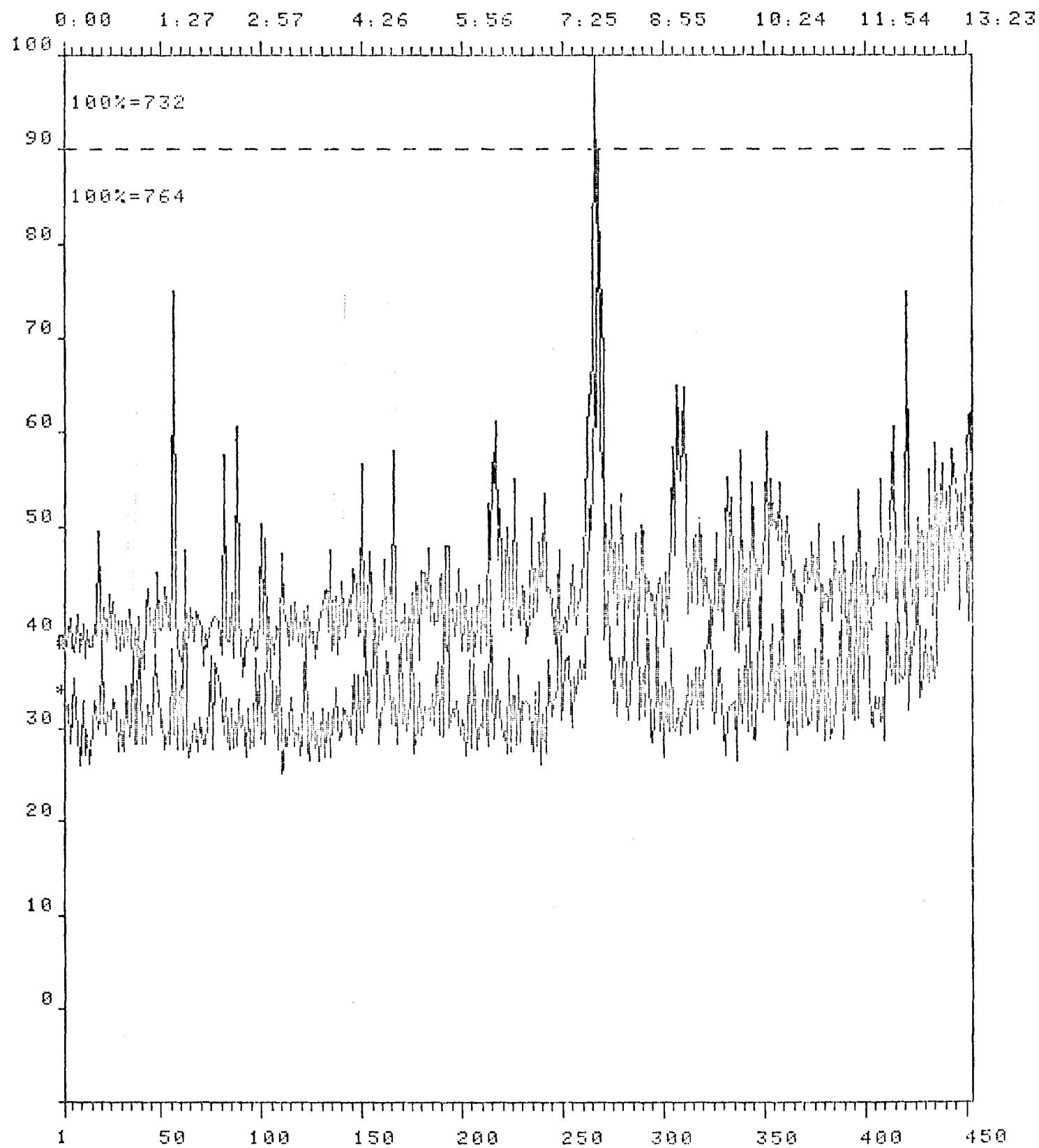
DS-55 CROSS SCAN REPORT, RUN: CWS60005

\* 374 # 376



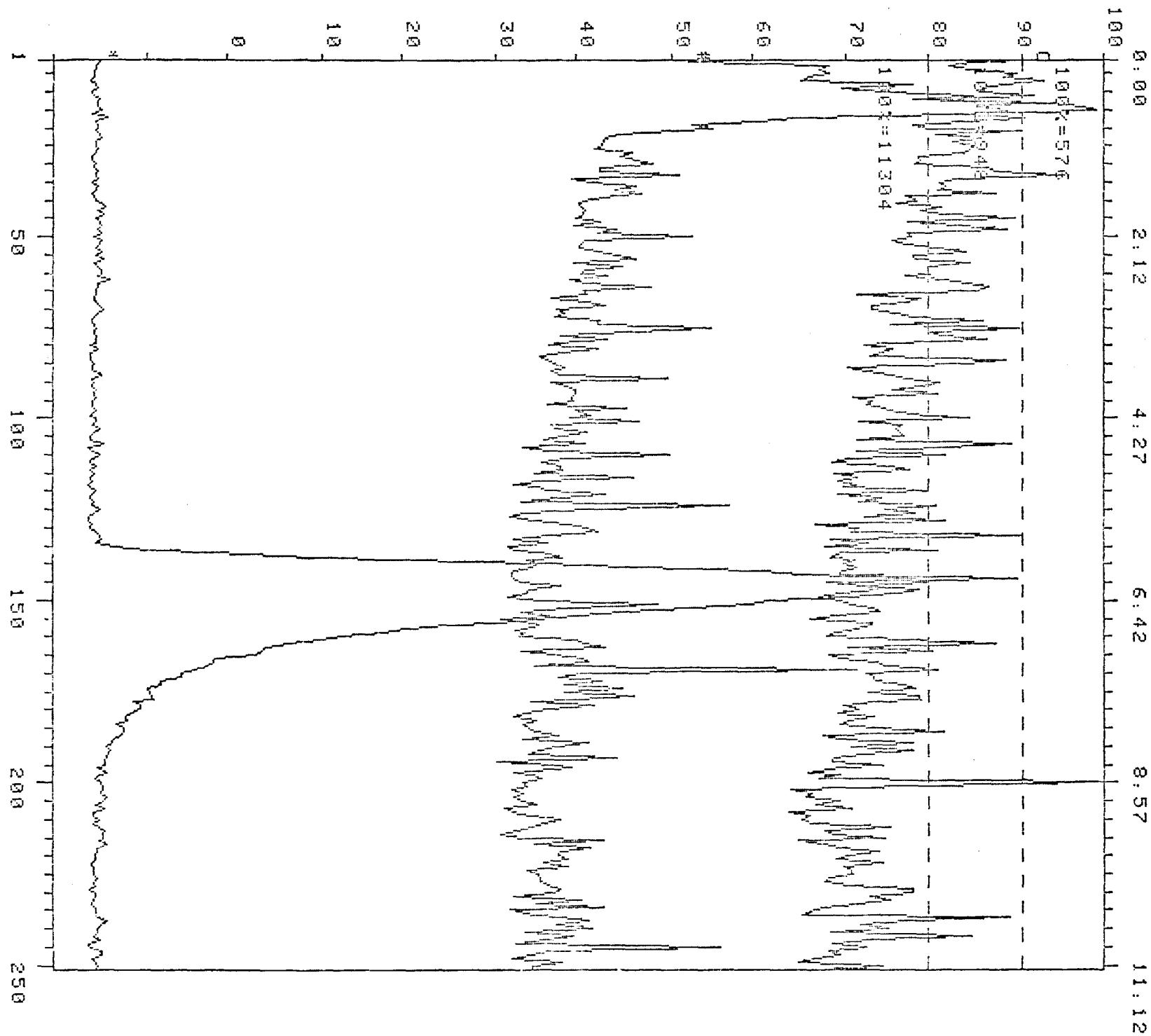
DS-55 CROSS SCAN REPORT, RUN: CMS60005

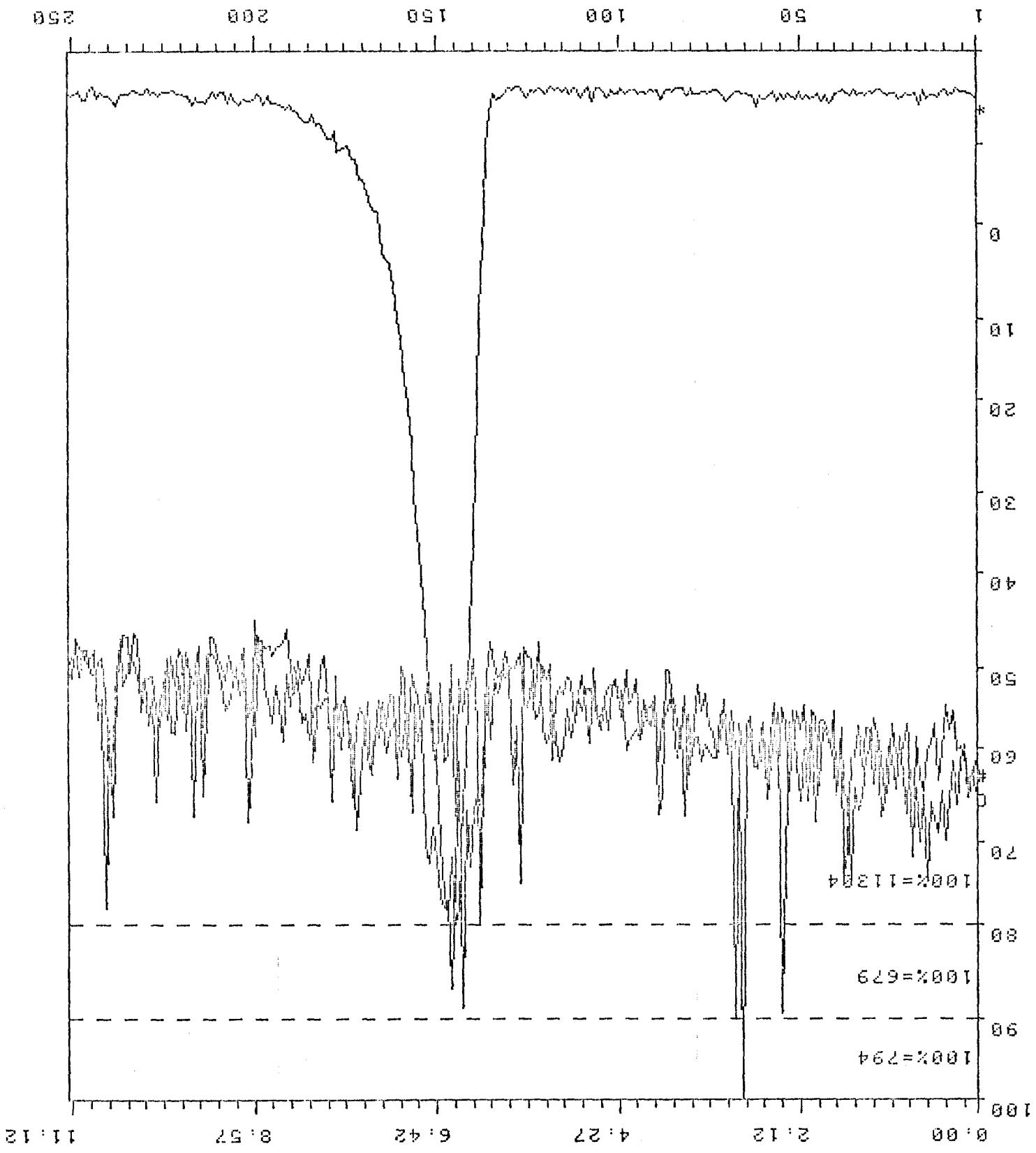
\* 390 # 392



DS-55 CROSS SCAN REPORT, RUN: CMS70005

\* 432 # 498 0 410



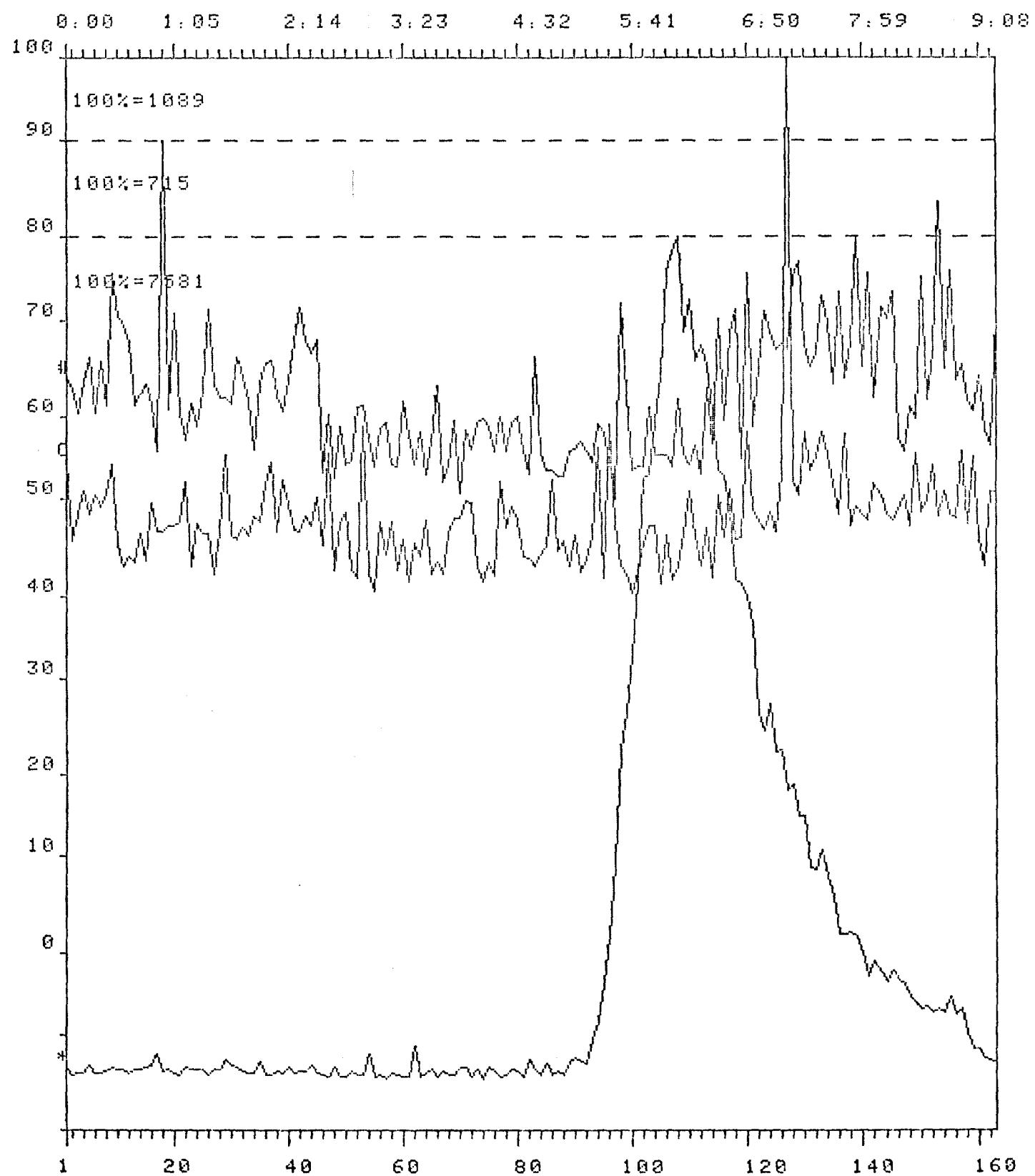


\* 432 # 424 O 426

DG-55 CROSS SCAN REPORT, RUM, QWS79005

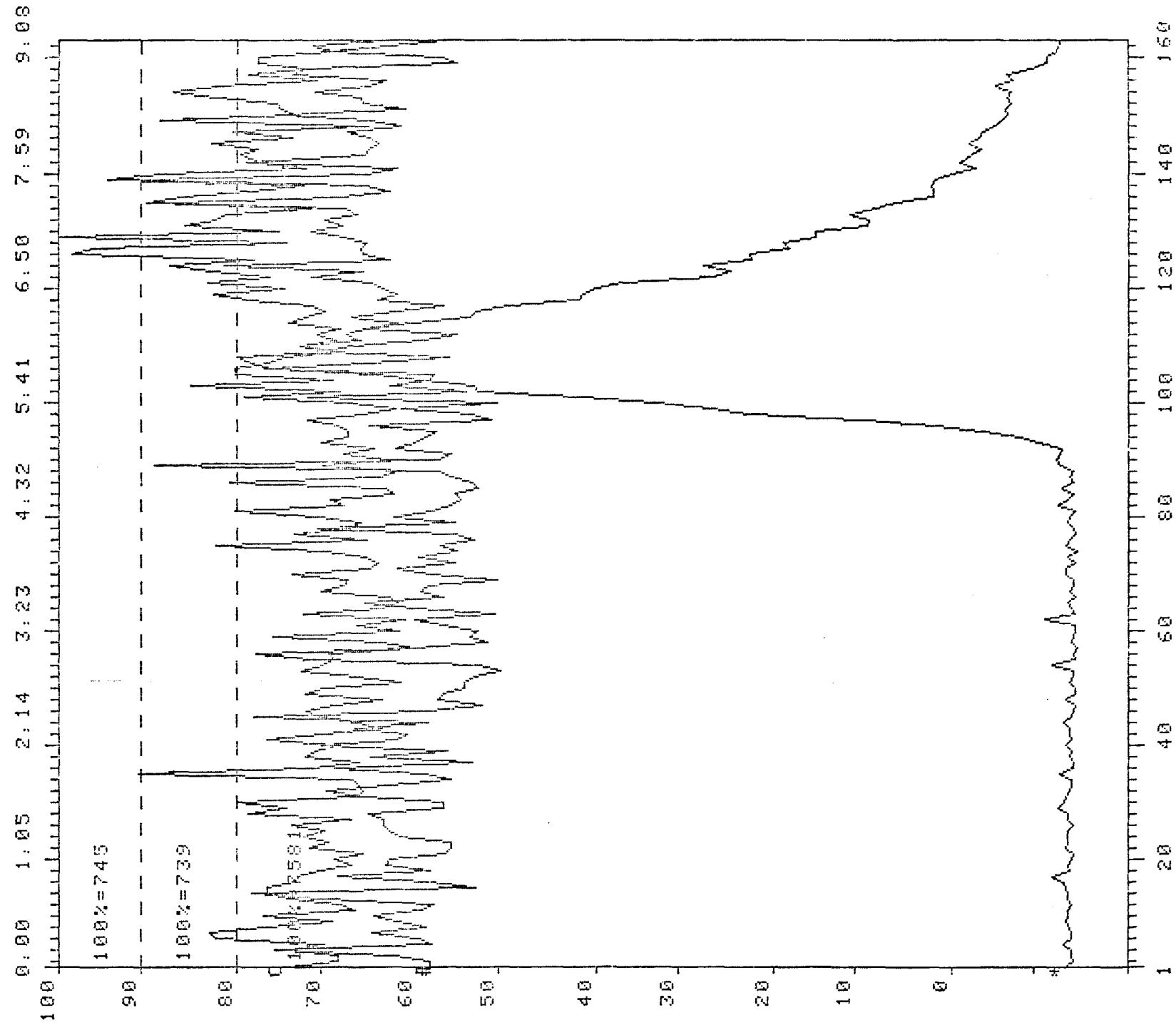
DS-55 CROSS SCAN REPORT, RUN: CWS80005

\* 472 # 442 0 444



DS-55 CROSS SCAN REPORT, RU4, CMS80005

\* 472 # 458 0 460

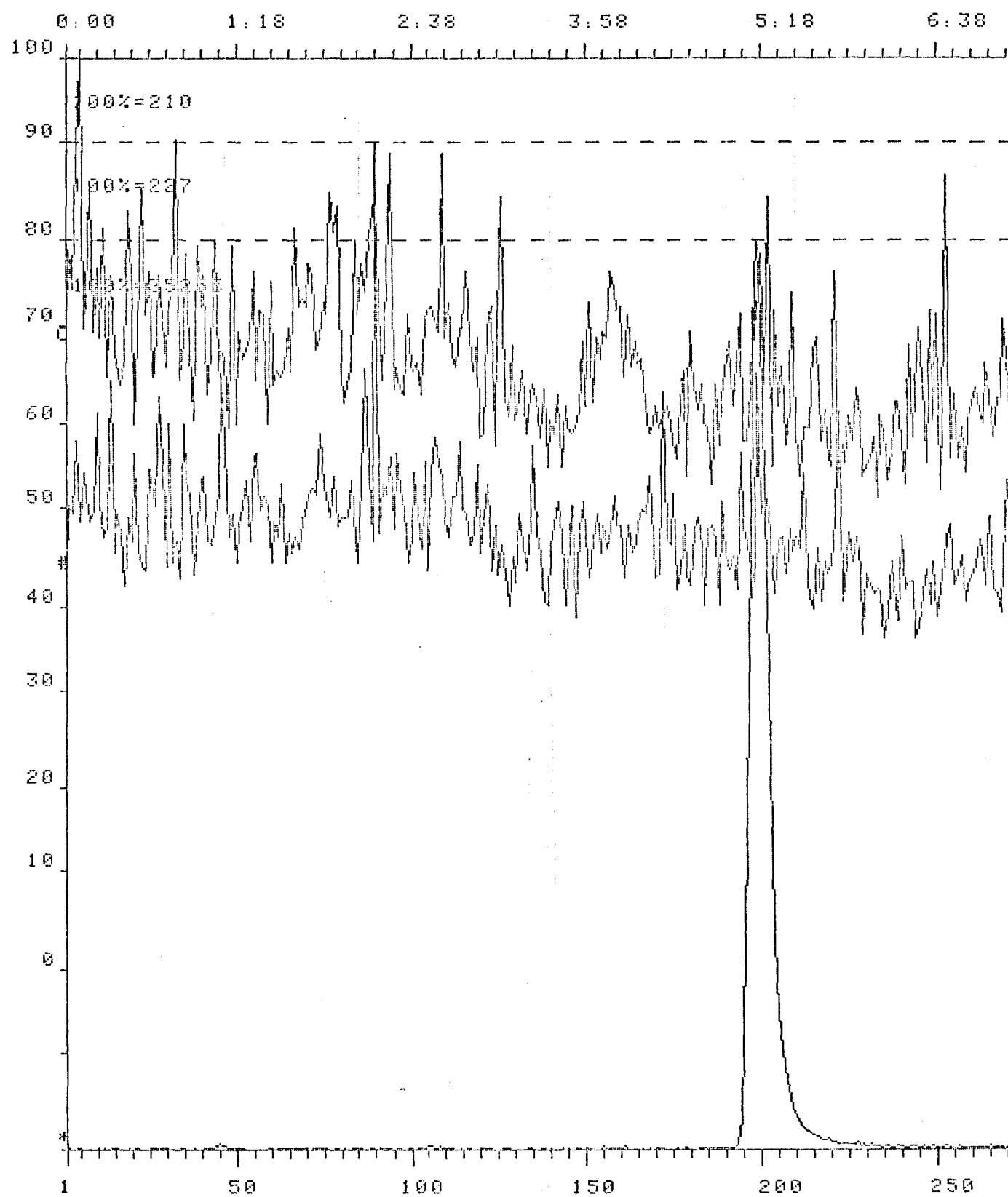


F. CWS-5

CWS-5

DS-55 CROSS SCAN REPORT, RUN: CW5B40002

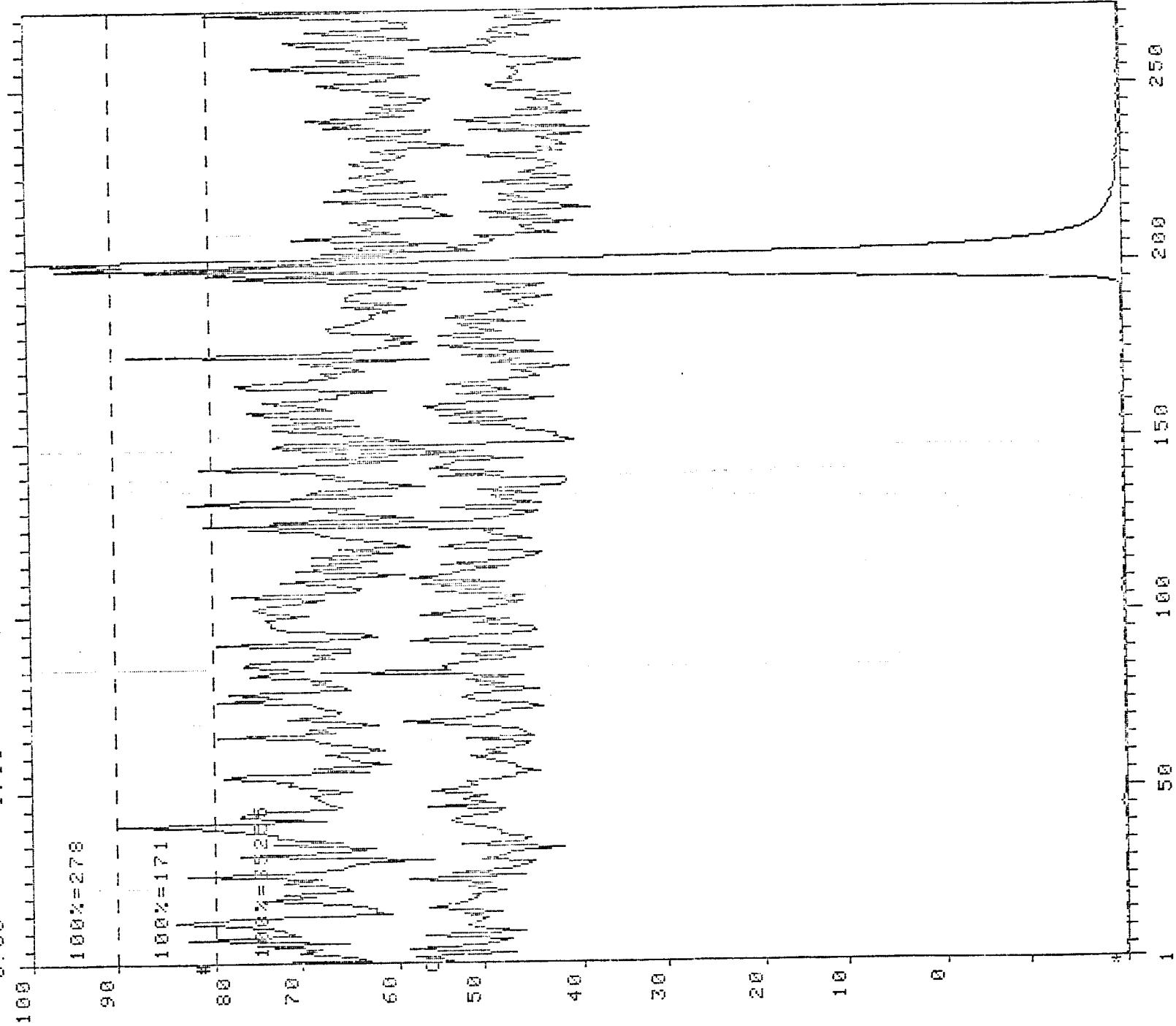
\* 328 # 304 O 306

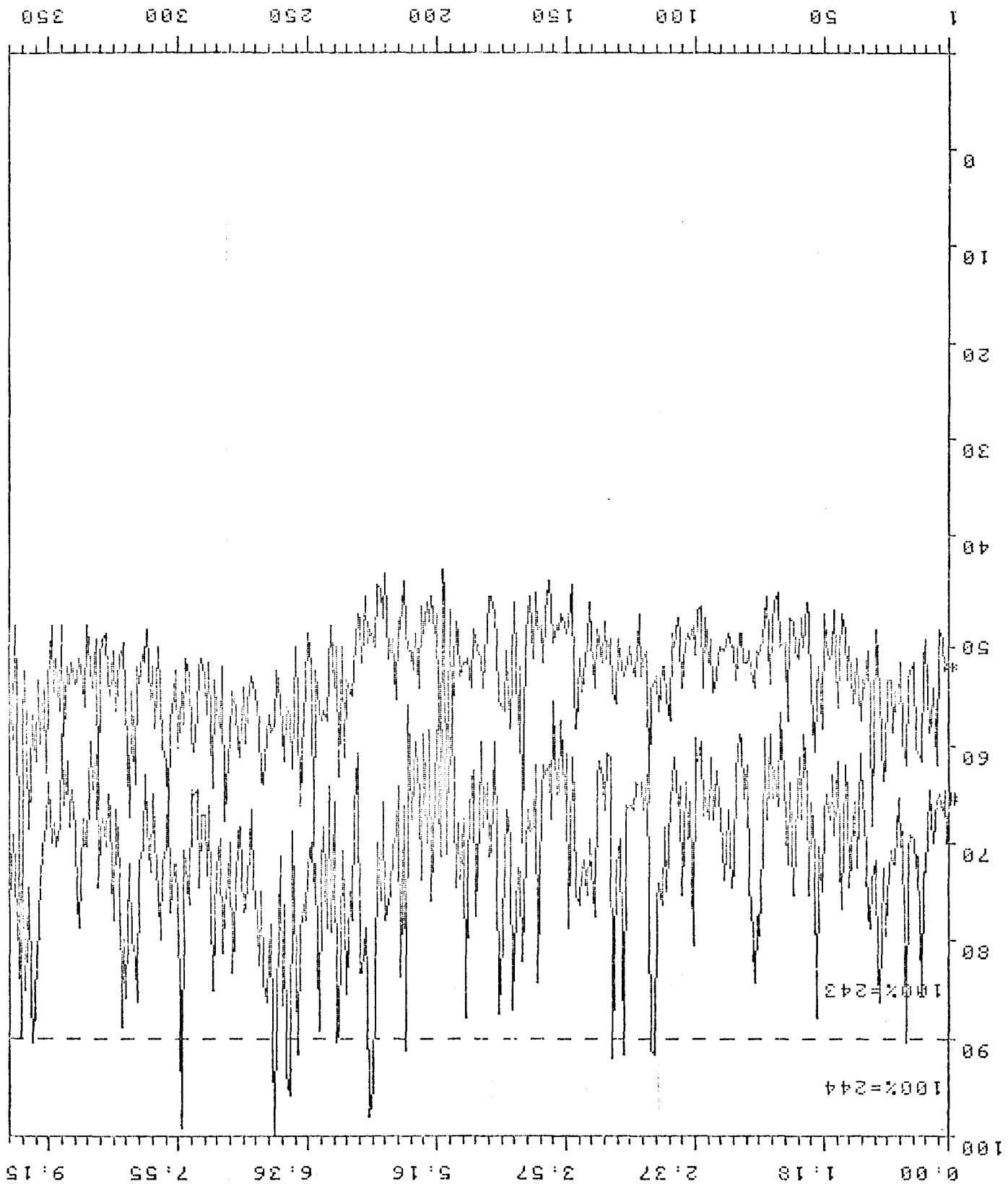


DS-55 CROSS SCAN REPORT, RUN: CWSB46002

\* 328 # 320 0 322

0 : 00 1 : 18 2 : 38 3 : 58 5 : 18 6 : 38



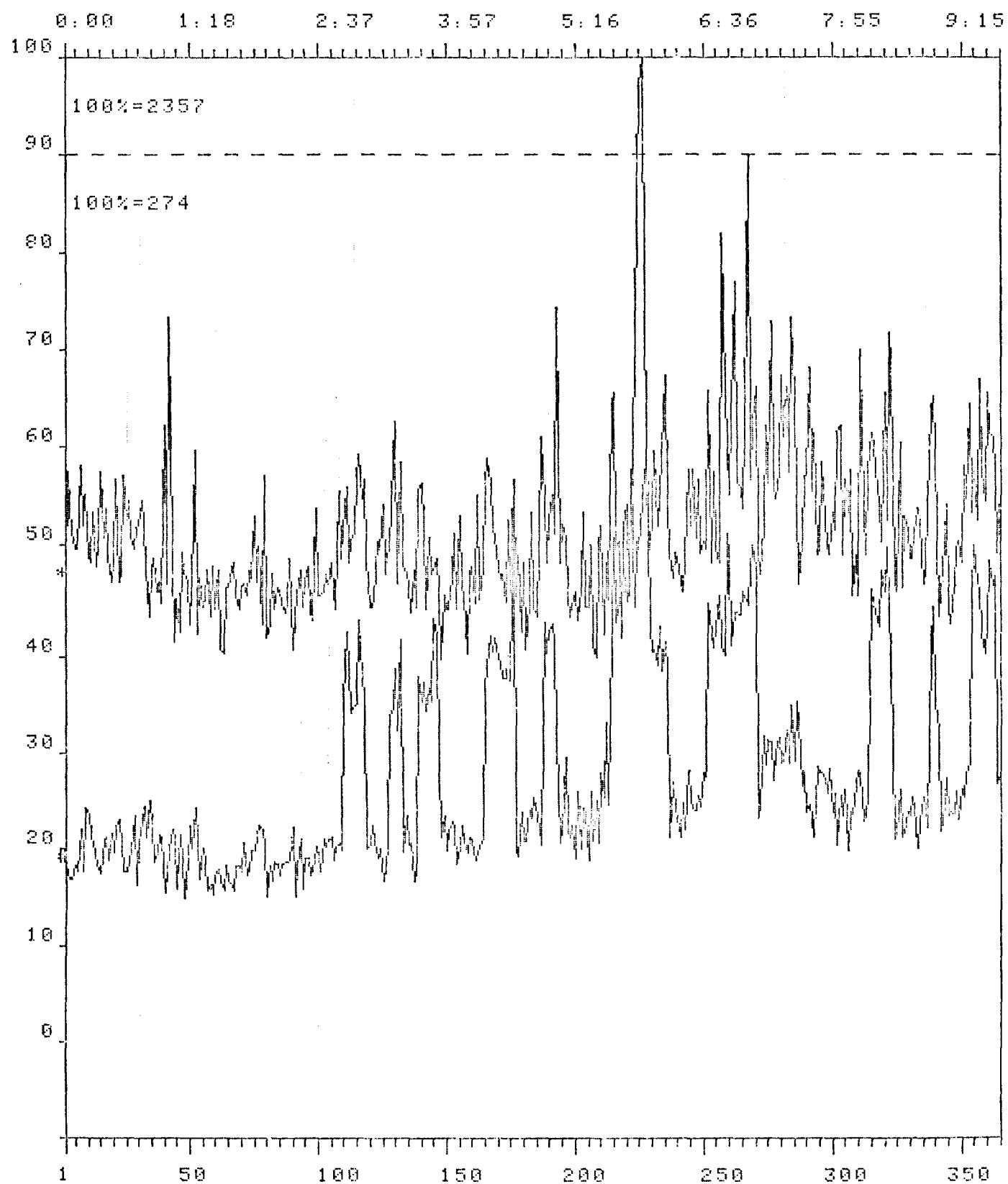


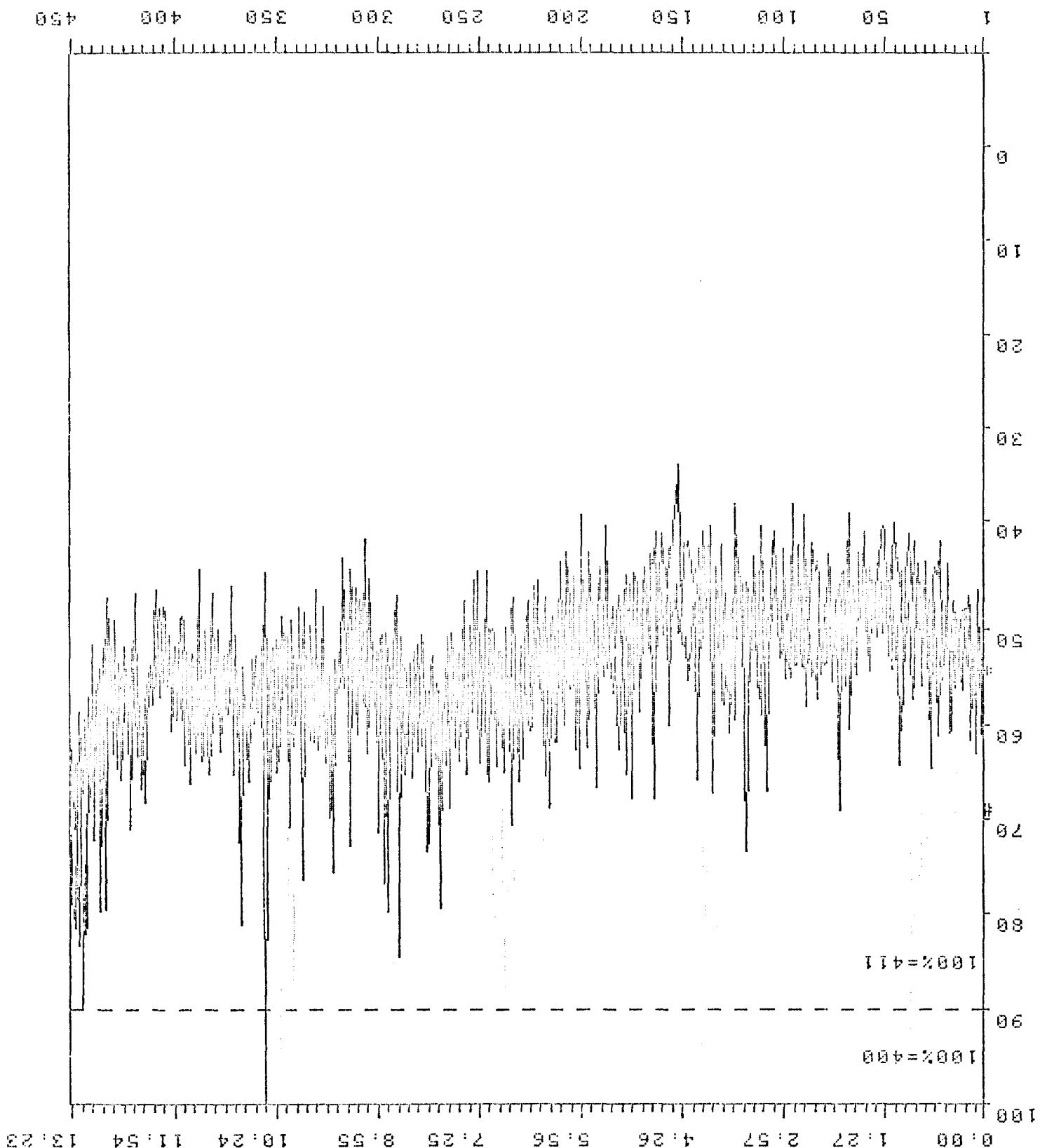
\* 328 # 340

DS-55 CROSS SCAN REPORT, RUN: CUS550004

DS-55 CROSS SCAN REPORT, RUN: CWS50004

\* 354 # 356



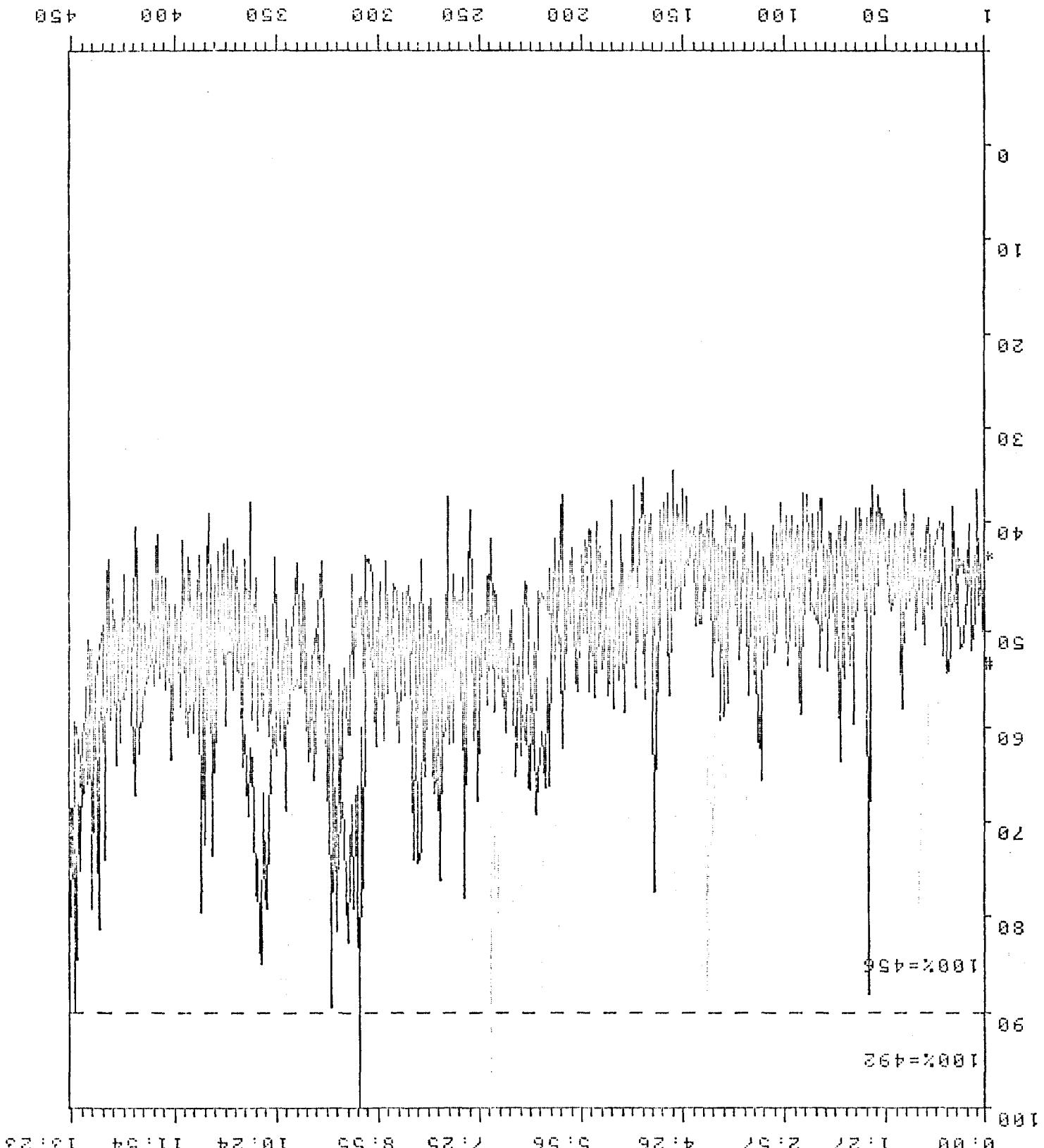


DS-55 CROSS SCAN REPORT, RUN: CWS60004

\* 374 # 376

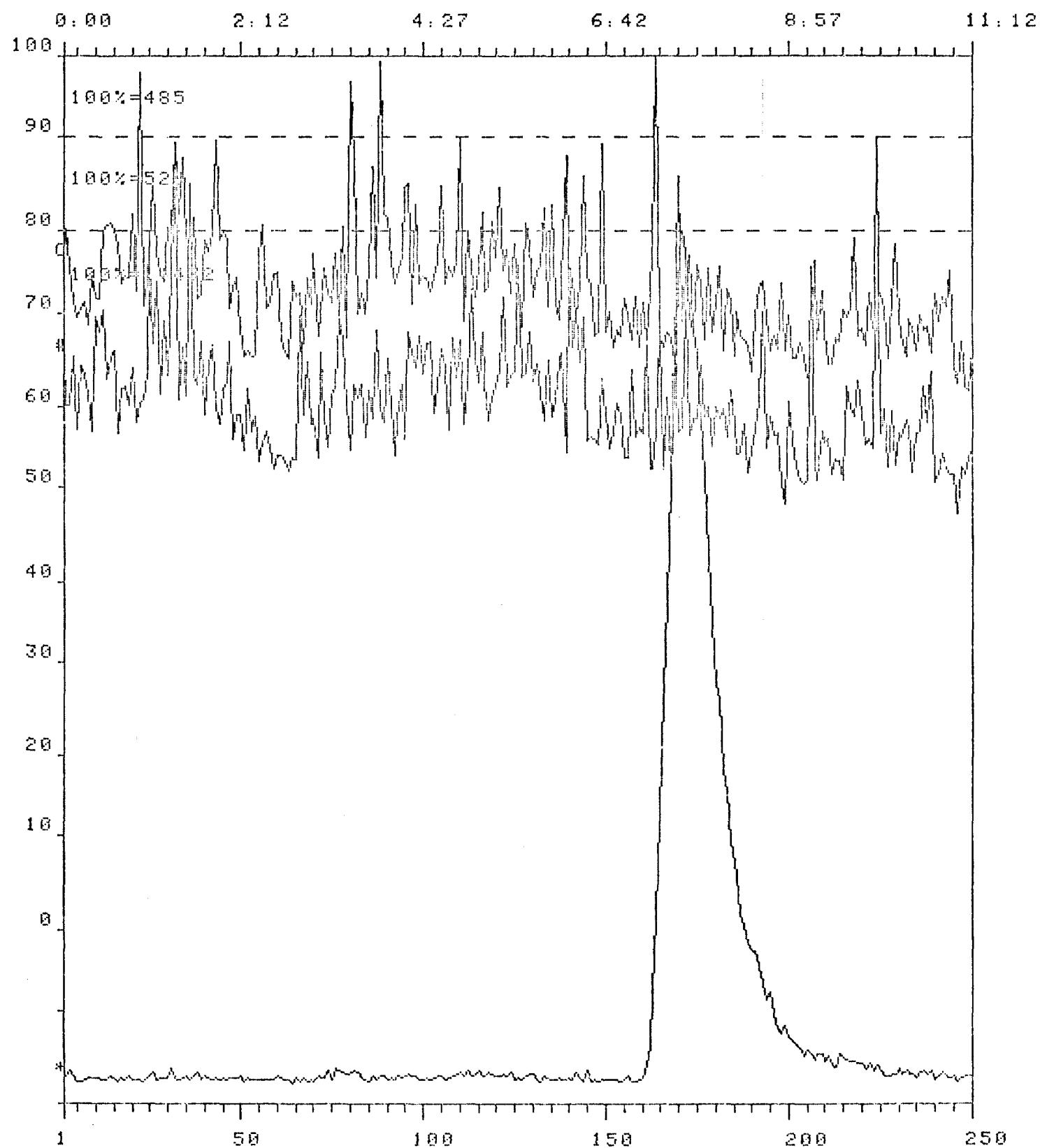
\* 398 # 392

DS-55 CROSS SCAN REPORT, RUN, CWS69004



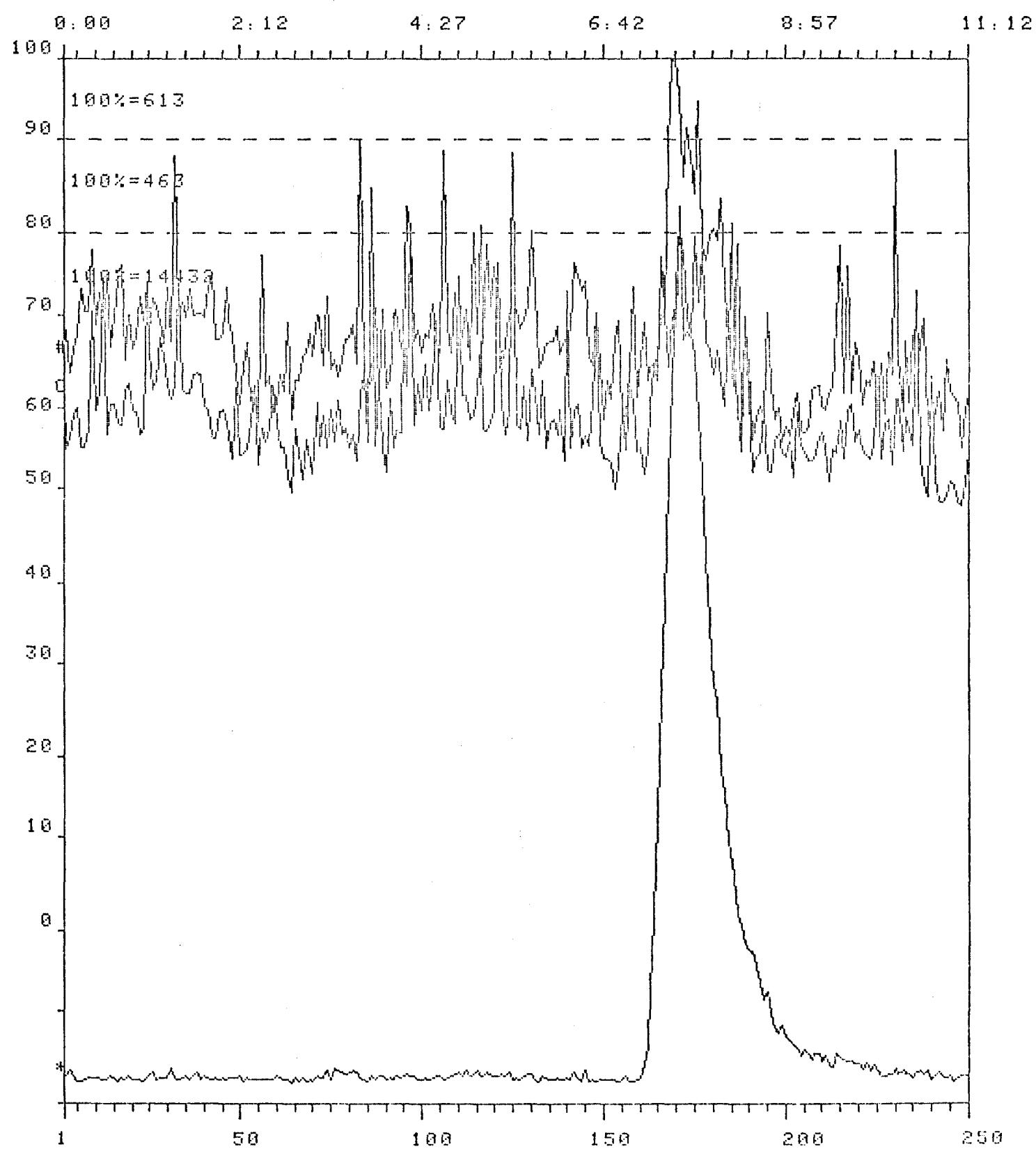
DS-55 CROSS SCAN REPORT, RUN: CWS70004

\* 432 # 408 O 410



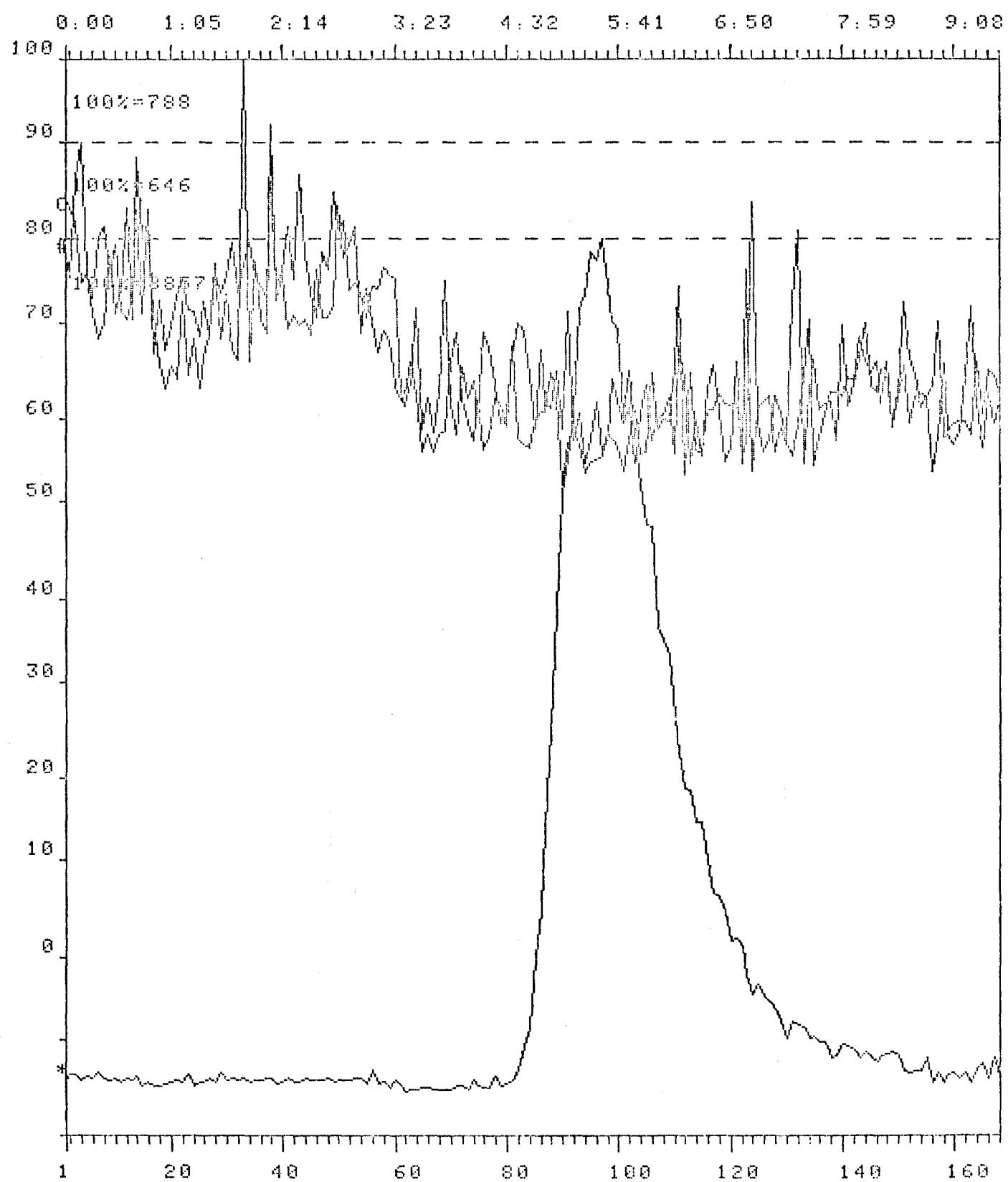
DS-55 CROSS SCAN REPORT, RUN: CWS70004

\* 432 # 424 0 426



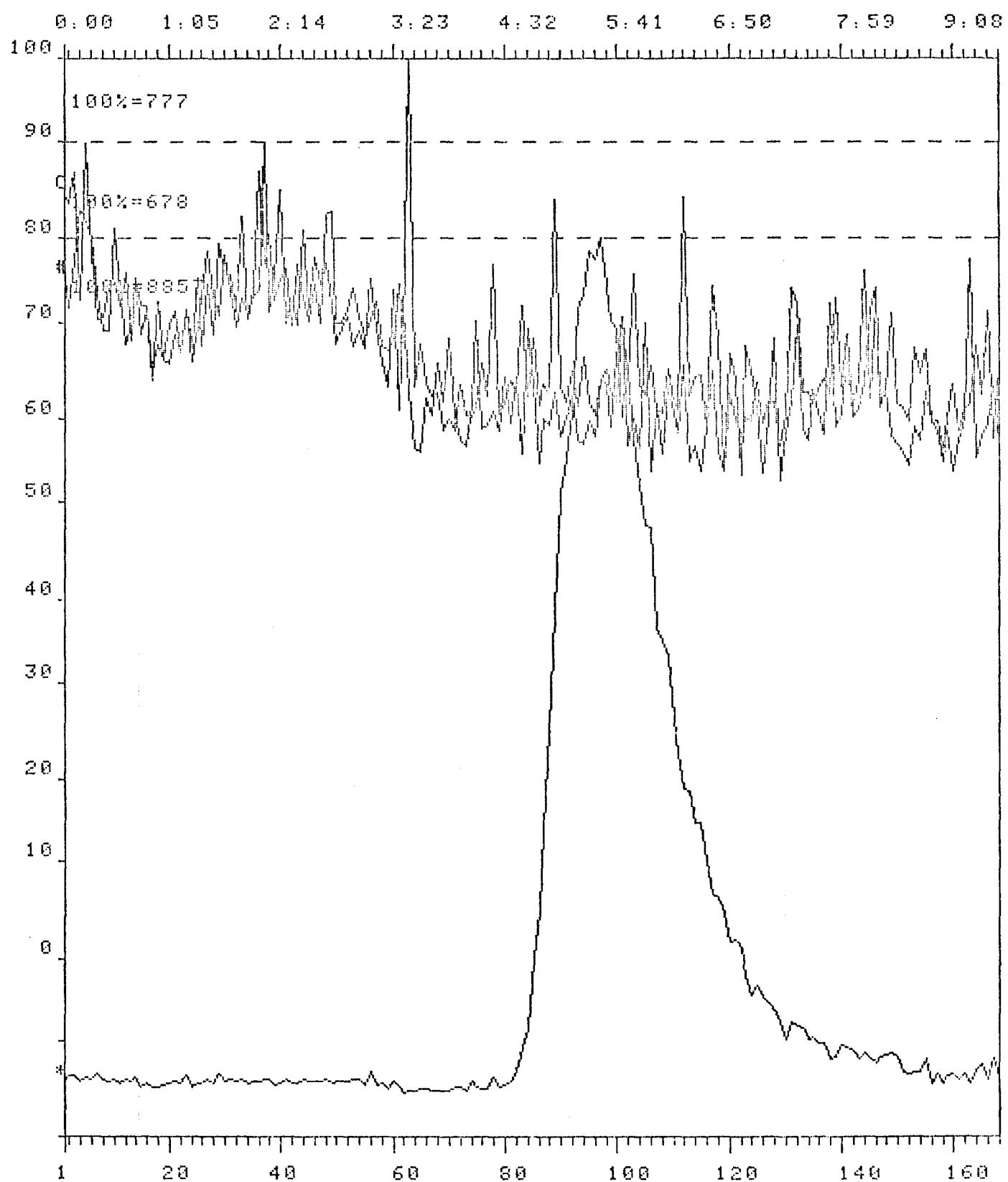
DS-55 CROSS SCAN REPORT, RUN: CWSS86004

\* 472 # 442 0 444



DS-55 CROSS SCAN REPORT, RUN: CWS80004

\* 472 # 458 O 460



ATTACHMENT 2

WRIGHT STATE UNIVERSITY, BREHM LABORATORY

PROTOCOL FOR ANALYSIS OF LEACHATE SAMPLES

BREHM LABORATORY, WRIGHT STATE UNIVERSITY, DAYTON, OHIO 45435

ANALYTICAL PROTOCOL FOR DETERMINATION OF CHLORINATED  
DIBENZO-p-DIOXINS AND CHLORINATED DIBENZOFURANS  
IN LEACHATE SAMPLES FROM HAZARDOUS LANDFILLS

A. Extraction and Preliminary Separation of CDDs and CDFs From Other Sample Matrix Constituents

1. Thoroughly agitate the sample to ensure complete mixing of the liquid phase and the fine particulate therein. Transfer 100 ml of the sample to a 250 ml flint glass bottle equipped with a Teflon-lined cap.
2. Add the following standards to each of the samples in the specified quantities: 10 ng of  $^{37}\text{Cl}_4\text{-}2,3,7,8\text{-TCDD}$ , 20 ng  $^{37}\text{Cl}_4\text{-HpCDD}$  and 36 ng  $^{37}\text{Cl}_8\text{-OCDD}$ . Agitate each bottle for 30 seconds.
3. Add 50 ml of hexane to the sample bottle and again agitate for a period of 1 hour. Allow the mixture to stand until complete separation of the aqueous and organic phases occurs. Remove and discard the aqueous (bottom) layer.
4. Extract the organic extract with 30 ml of 20% (w/v) potassium hydroxide by agitating with the extract for 10 minutes. Allow the mixture to stand for a period sufficient for complete separation of the aqueous and organic layers, and remove and discard the aqueous base (bottom) layer.
5. Extract the organic extract with 30 ml of doubly distilled water by agitating with the extract for 2 minutes. Allow time for complete layer separation, and remove and discard the aqueous (bottom) layer.
6. Extract the organic extract with 30 ml of sulfuric acid by agitating with the extract for 2 minutes. Allow for complete layer separation and remove and discard the acid (bottom) layer.
7. Extract the organic extract with 30 ml of sulfuric acid by agitating with the extract for 2 minutes. Allow for complete layer separation and remove and discard the aqueous (bottom) layer.

8. Dry the organic extract over sodium sulfate and then quantitatively transfer it to a clean test tube and reduce the volume to incipient dryness using a stream of prepurified nitrogen, while maintaining the test tube in a 55° water bath.
9. Fabricate a glass Macro-column (20 mm OD x 230 mm long) tapered to 6 mm OD on one end. Pack the column sequentially with 1.0 g silica, 2.0 g silica containing 33% (w/w) 1 M NaOH, and 2.0 g silica.
10. Quantitatively transfer the residue obtained in Step 10 to the column and elute the column with 90 mL of hexane. Collect the eluent and concentrate to 1-2 mL in a centrifuge tube.
11. Construct a disposable liquid chromatography column as follows. Cut off a Pyrex 5 mL disposable pipet at the 2 mL mark and use the lower portion of the pipet. Pack the small end with a plug of silanized glass wool. Next, add 1 gram of Wöelm basic alumina previously activated overnight at 600°C in a muffle furnace and placed in a dessicator for 30 minutes just prior to use.
12. Using a disposable pipet, transfer the sample onto the liquid chromatography column.
13. Rinse the centrifuge tube with two consecutive 0.5 mL portions of 3% CH<sub>2</sub>Cl<sub>2</sub>-in-hexane, and transfer the rinses to the alumina column.
14. Elute the column with 4 mL of 3% (v/v) CH<sub>2</sub>Cl<sub>2</sub>-in-hexane and discard the eluent (taking care not to let the column run dry).
15. Elute the column with 30 mL of 50% (v/v) CH<sub>2</sub>Cl<sub>2</sub>-in-hexane, and retain the eluent for analysis.
16. Concentrate the solution to approximately 1 mL, using a stream of prepurified nitrogen as before. Rinse the centrifuge tube wall with an additional 1 mL of CH<sub>2</sub>Cl<sub>2</sub> and reconcentrate.
17. Quantitatively transfer the residue (using methylene chloride) to a 2 mL micro-reaction vessel.
18. Evaporate the solution in the micro-reaction vessel almost to dryness as previously, rinse the walls of the vessel with approximately 0.5 mL CH<sub>2</sub>Cl<sub>2</sub>, and evaporate contents just to dryness, storing the extract in a freezer until just prior to analysis.
19. Approximately 1 hour before GC-MS (GC-LRMS or GC-HRMS) analysis, dilute the residue in the micro-reaction vessel with an appropriate quantity of tridecane. Gently swirl the tridecane on the lower portion of the vessel to ensure dissolution of the chlorodioxin and chlorodibenzofurans. Inject an appropriate aliquot of the sample into the GC-MS instrument using a syringe.

20. If upon preliminary GC-MS analysis the sample appears to contain interferences which obscure the analyses for CDDs and CDFs, then high performance liquid chromatographic (HPLC) cleanup of the extract is accomplished at this point, prior to further GC-MS analysis. For HPLC, a Varian Model 5021 instrument, equipped with a DuPont Zorbax-ODS (C-18, reverse phase) column, is employed.

B. Procedures and Operating Parameters for High Performance Liquid Chromatographic and Gas Chromatographic-Mass Spectrometric Analysis of Extracts

1. High Performance Liquid Chromatography Procedures

a. Instrumentation: Varian Model 5021 Microprocessor Controlled High Performance Chromatograph equipped with CDS-111L Data System

b. Parameters:

Pressure: Minimum: 10 atm

Maximum: 250 atm

Injection Loop: 50 l

Column: Guard: 37 Vydac SC Reverse Phase

4.0 cm x 0.4 cm I.D.

Analytical: 2-DuPont Zorbax-ODS

25.0 cm x 0.6 cm I.D.

Temperature: Guard Column: Ambient

Analytical Column: 50°C

Detector: Fixed UV: 254nm, 0.01 A.U.F.S.

Variachrom UV-Vis: Mono-Penta CDDs and CDFs 235nm

Hexa-Octa CDDs and CDFs 245nm

0.01 A.U.F.S.

Program:	Time	Code	Value
	.0	%	100 Methanol
	.0	Flow	2.5 mL/min
	.0	Event	Hold
	.1	Event	Inject
	20.0	Event	Reset

**c. HPLC Collection Procedure for CDDs and CDFs**

1. Place approximately 2 mL of hexane in a 50 mL flint glass bottle fitted with a Teflon-lined cap.
  2. At the appropriate retention time, position sample bottle to collect the required fraction.
  3. Add 2 mL of 5% (w/v) sodium carbonate to the sample fraction collected and shake for one minute.
  4. Quantitatively remove the hexane layer (top layer) and transfer to a micro-reaction vessel.
  5. Concentrate the fraction to dryness and retain for further analysis.
2. High Resolution Gas Chromatographic Low Resolution Mass Spectrometric (GC-LRMS) Procedures For Analysis of the Extracts

a. Instrumentation: Perkin Elmer Sigma III Gas Chromatograph coupled through a custom-fabricated interface including a single-stage glass jet separator to a Kratos MS-25 Mass Spectrometer equipped with a DS-50 SM Data System.

b. Conditions for the Gas Chromatograph:

Column: 50M WCOT (OV-101) Silica Capillary

Carrier Gas: Hydrogen, 30 lb. head pressure

Column Temperature: Isothermal at 190° for 2 minutes, Programmed at 5°C/min from 190°C to 220°C, hold at 220°C for 17 min, then program at 5°C/min to 230°C, hold at 230°C for 23 min, then program at 5°C/min to 235°C, hold at 235°C for 78 min.

Interface Temperature: 250°C

Injector: Splitless Injection

c. Conditions for the Mass Spectrometer:

Operate In Selected Ion Monitoring Mode (Procedures and m/z's monitored are shown in Tables 1-3)

Ionizing Voltage: 70 eV

Accelerating Voltage: 4 KV

**C. Reagents and Chemicals**

The following is a listing of the reagents and chemicals utilized in the

procedures outlined above. Potassium hydroxide, anhydrous sodium sulfate, and sulfuric acid were all Reagent Grade and were obtained from J.T. Baker Chemical Co. or Fisher Scientific Co., Fairlawn, N.J. Methanol, hexane, methylene chloride and benzene were "Distilled in Glass" quality obtained from Burdick and Jackson, Muskegon, Michigan. Wöelm basic alumina (Activity Grade I) was obtained from ICN Pharmaceuticals, Cleveland, Ohio. Silica (Bio-Sil A) was obtained from Bio-Rad, Richmond, CA. Doubly distilled water was obtained using an all-glass distillation apparatus in the Brehm Laboratory. Prepurified nitrogen was obtained from Airco, Inc., Montvale, New Jersey. Standards employed in this work were obtained from the following sources;  $^{37}\text{Cl}_4$ -2,3,7,8-TCDD, KOR Isotopes, Cambridge, Mass.; OCDD, Analabs; 2,3,7,8-tetrachlorodibenzofuran and OCDF, U.S. Food and Drug Administration, Washington, D.C.

Chlorodioxin and chlorinated dibenzofuran standards are listed in Tables 4 and 5.

TABLE 1

WRIGHT STATE UNIVERSITY, BREHM LABORATORY, DAYTON, OHIO 45435

LIST OF ION MASSES MONITORED USING GC-SELECTED-ION MONITORING MASS SPECTROMETRY FOR SIMULTANEOUS  
DETERMINATION OF MONO-, DI-, TRI-, TETRA-, PENTA-, HEXA-, HEPTA-, and OCTA-, CHLORINATED DIBENZO-  
p-DIOXINS and DIBENZOFURANS

<u>Class of Chlorinated Dibenzodioxin or Dibenzofuran</u>	<u>Number of Chlorine Substituents (X)</u>	<u>Monitored m/z for Dibenzofurans <math>C_{12}H_{8-x}OCl_x</math></u>	<u>Monitored m/z for Dibenzo-p-dioxins <math>CH_{12}H_{8-x}O_2Cl_x</math></u>	<u>Approximate Theoretical Ratio Expected on Basis of Isotopic Abundance</u>
Mono	1	202.019 <sup>a</sup> 204.016	218.013 <sup>a</sup> 220.011	1.00 0.35
Di	2	235.980 <sup>a</sup> 237.977	251.974 <sup>a</sup> 253.972	1.00 0.69
Tri	3	269.941 <sup>a</sup> 271.938	285.940 <sup>a</sup> 287.937	0.99 1.00
Tetra	4	303.902 <sup>a</sup> 305.899	319.897 <sup>a</sup> 321.894 <sup>b</sup> 327.885 [256.933] <sup>c</sup> 258.930	0.74 1.00 -- 0.21 0.20
Penta	5	337.863 <sup>a</sup> 339.860	353.858 <sup>a</sup> 355.855	0.57 1.00
Hexa	6	373.821 375.818	389.816 391.813	1.00 0.87
Hepta	7	407.872 409.779	423.777 425.774 431.765 <sup>b</sup>	1.00 1.00 --
Octa	8	441.743 443.740	457.738 459.735 471.717 <sup>b</sup>	0.86 1.00

a. Molecular ion peak. b.  $^{37}Cl$ -labelled standard peaks. c. Ions which can be monitored in TCDD analyses for confirmation purposes.

TABLE 2

SEQUENCE OF OPERATIONS IN GC-MS (MS-25) ANALYSES OF  
CHLORODIBENZODIOXINS AND CHLORODIBENZOFURANS  
IN FIRST INJECTION OF SAMPLE EXTRACT

<u>Elapsed Time (Min)</u>	<u>Event</u>	<u>GC Column Temperature</u>	<u>Temperature Program Rate (°C/MIN)</u>	<u>Ions Monitored By Mass Spectrometer (m/z)</u>	<u>Compounds Monitored</u>
0.00	Injection, splitless	190°C	--	--	--
1.50	Turn on split valve	190°C	--	--	--
2.00	Begin Temp. program to 220°C	190°C	5°C/min	--	--
4.50	Open column flow to Mass Spec.	202°C	5°C/min	--	--
5.00	Start PROGRAM #1  Sweep = 100 ppm  Time on each mass = 0.15 sec	205°C	5°C/min	202.019 204.016 218.013 220.011	Mono-Cl furans Mono-Cl furans Mono-Cl dioxins Mono-Cl dioxins
8.00	Column reaches isothermal hold	220°C	--	--	--
8.75	Stop PROGRAM #1	220°C	--	--	--
9.50	Start PROGRAM #3  Sweep = 100 ppm  Time on each mass = 0.15 sec	220°C	--	269.941 271.938 285.940 289.937	Cl <sub>3</sub> furans Cl <sub>3</sub> furans Cl <sub>3</sub> dioxins Cl <sub>3</sub> dioxins
16.00	Stop PROGRAM #3				
16.75	Start PROGRAM #5  Sweep = 125 ppm  Time on penta mass = 0.2 sec  Time on <sup>37</sup> Cl-TCDD = 0.07 sec	220°C	--	327.885 337.863 339.860 353.858 355.855	<sup>37</sup> Cl labelled TCDD Cl <sub>5</sub> furans Cl <sub>5</sub> furans Cl <sub>5</sub> dioxins Cl <sub>5</sub> dioxins
25.00	Begin temp program to 235°C	220°C	5°C/sec	--	--
28.00	Stop PROGRAM #5	235°C	--	--	--

TABLE 2 (cont)

<u>Elapsed Time (Min)</u>	<u>Event</u>	<u>GC Column Temperature</u>	<u>Temperature Program Rate (<math>^{\circ}</math>C/Min)</u>	<u>Ions Monitored By Mass Spectrometer (m/z)</u>	<u>Compounds Monitored</u>
45.00	Start PROGRAM #7 Sweep = 175 ppm Time on each mass = 0.35 sec	235 $^{\circ}$ C	--	407.782 409.779 423.777 425.774 431.765	Cl <sub>7</sub> furans Cl <sub>7</sub> furans Cl <sub>7</sub> dioxins Cl <sub>7</sub> dioxins <sup>37</sup> Cl labelled HpCDD
60.00	Stop PROGRAM #7		--	--	--
95.00	Return GC to initial temp		--	--	--

TABLE 3

SEQUENCE OF OPERATIONS IN GC-MS (MS-25) ANALYSES OF  
CHLORODIBENZODIOXINS AND CHLORODIBENZOFURANS  
IN SECOND INJECTION OF SAMPLE EXTRACT

<u>Elapsed Time (Min)</u>	<u>Event</u>	<u>GC Column Temperature</u>	<u>Temperature Program Rate ( °C/Min)</u>	<u>Ions Monitored By Mass Spectrometer (m/z)</u>	<u>Compounds Monitored</u>
0.00	Injection, splitless	190°C	--	--	--
1.50	Turn on split valve	190°C	--	--	--
2.00	Begin temp program to 220°C	190°C	--	--	--
4.50	Open flow to Mass Spec.	202°C	5°C/min		
6.00	Start PROGRAM #2 Sweep = 100 ppm Time on each mass = 0.15 sec	210°C	5°C/min	235.980 237.977 251.974 253.972	Cl <sub>2</sub> furans Cl <sub>2</sub> furans Cl <sub>2</sub> dioxins Cl <sub>2</sub> dioxins
8.00	Column reaches 220°C hold isothermal	220°C	--	--	--
12.00	Stop PROGRAM #2	220°C	--	--	--
12.75	Start PROGRAM #4 Sweep - 100 ppm Time on Cl mass = 0.15 sec Time on Cl-TCDD = 0.05 sec	220°C	--	303.902 305.899 319.897 321.894 327.885	Cl <sub>4</sub> furans Cl <sub>4</sub> furans Cl <sub>4</sub> dioxins Cl <sub>4</sub> dioxins <sup>37</sup> Cl labelled TCDD
24.00	Stop PROGRAM #4	220°C	--	--	--
25.00	Begin temp program to 235°C	220°C	5°C/min	--	--
26.00	Start PROGRAM #6 Sweep = 150 ppm Time on each mass = 0.25 sec	225°C	5°C/min	373.821 375.818 389.816 391.813	Cl <sub>6</sub> furans Cl <sub>6</sub> furans Cl <sub>6</sub> dioxins Cl <sub>6</sub> dioxins
45.00	Stop PROGRAM #6	235°C	--	--	--
46.00	Start PROGRAM #7 Sweep = 175 ppm Time on each mass = 0.035 sec	235°C	--	407.782 409.779 423.777 425.774 431.765	Cl <sub>7</sub> furans Cl <sub>7</sub> furans Cl <sub>7</sub> dioxins Cl <sub>7</sub> dioxins <sup>37</sup> Cl labelled HpCDD
60.00	Stop PROGRAM #7	235°C	--	411.743	Cl <sub>8</sub> furans
70.00	Start PROGRAM #8 Sweep = 225 ppm Time on each mass = 0.55 sec	235°C	--	443.740 457.738 459.735	Cl <sub>8</sub> furans Cl <sub>8</sub> dioxins Cl <sub>8</sub> dioxins
90.00	Stop PROGRAM #8	235°C	--		
95.00	Return to initial temp				

TABLE 4

LIST OF CHLORODIOXIN ISOMER STANDARDS CURRENTLY AVAILABLE  
AT THE BREHM LABORATORY-WRIGHT STATE UNIVERSITY

1-Chlorodibenzo-p-dioxin

2-Chlorodibenzo-p-dioxin

2,7-Dichlorodibenzo-p-dioxin

2,3-Dichlorodibenzo-p-dioxin

1,2,4-Trichlorodibenzo-p-dioxin

Tetrachlorodibenzo-p-dioxins - all 22 isomers

$^{37}\text{Cl}_4$ -2,3,7,8-Tetrachlorodibenzo-p-dioxin

$^{13}\text{C}_4$ -2,3,7,8-Tetrachlorodibenzo-p-dioxin

$^{14}\text{C}_4$ -2,3,7,8-Tetrachlorodibenzo-p-dioxin

1,2,3,7,8-Pentachlorodibenzo-p-dioxin

1,2,4,6,7,9-Hexachlorodibenzo-p-dioxin

1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin

1,2,3,4,6,7-Hexachlorodibenzo-p-dioxin

1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin

$^{37}\text{Cl}_4$ -1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin

Octachlorodibenzo-p-dioxin

$^{37}\text{Cl}_8$ -Octachlorodibenzo-p-dioxin

TABLE 5

LIST OF CHLORINATED DIBENZOFURAN STANDARDS CURRENTLY AVAILABLE  
AT THE BREHM LABORATORY-WRIGHT STATE UNIVERSITY

**2,4-Dichlorodibenzofuran**

**3,6-Dichlorodibenzofuran**

**2,8-Dichlorodibenzofuran**

**1,2,4-Trichlorodibenzofuran**

**1,2,4,8-Tetrachlorodibenzofuran**

**2,3,7,8-Tetrachlorodibenzofuran**

**1,2,4,7,8-Pentachlorodibenzofuran**

**1,2,4,6,7,9-Hexachlorodibenzofuran**

**1,2,3,4,6,8,9-Heptachlorodibenzofuran**

**Octachlorodibenzofuran**

**<sup>37</sup>Cl<sub>4</sub>-2,3,7,8-Tetrachlorodibenzofuran**